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# British Journal of Obstetrics and Gynaecology

(**formerly** JOURNAL OF OBSTETRICS AND GYNAECOLOGY
OF THE BRITISH COMMONWEALTH)

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[1] *Postgrad med J*, (1971) **47**, Supplement (September), 7.
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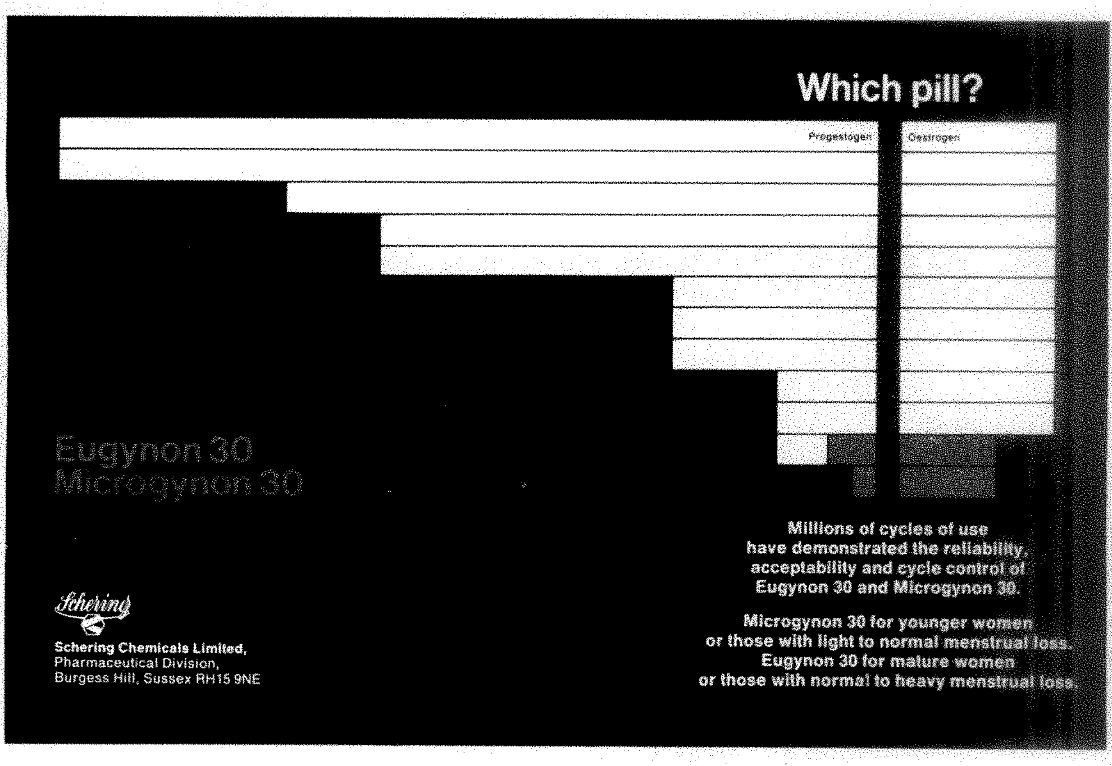

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**References** 1. Proost, et al., *Am. J. Obst. & Gynae., (Drug Res.)* **21:** 256-257, (1971) 2. Fahlberg, W.J., and Marcuse, P.M., (ed.): *Diagnostic Pathology in Gynaecology and Obstetrics*, New York, Harper & Row, 1966, pp. 319-321.

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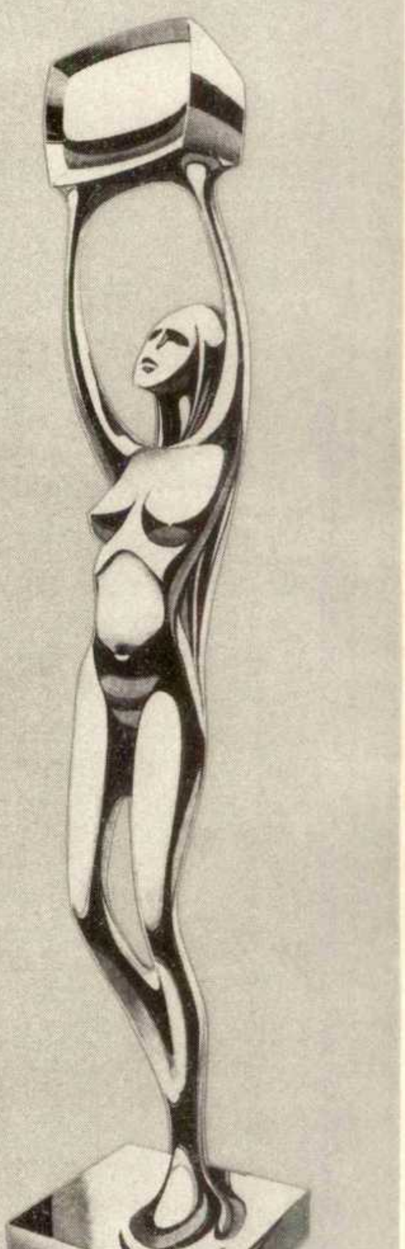

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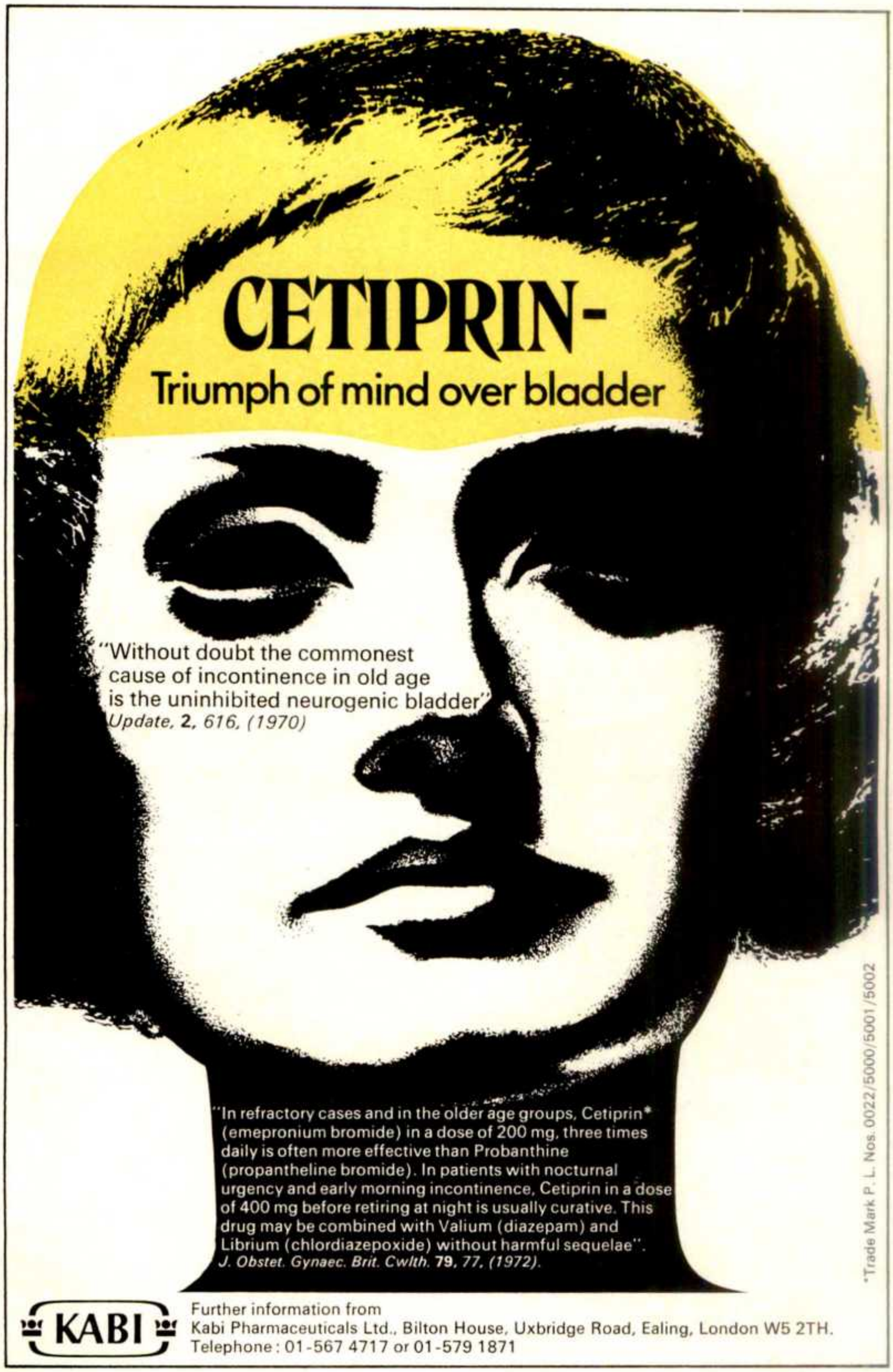

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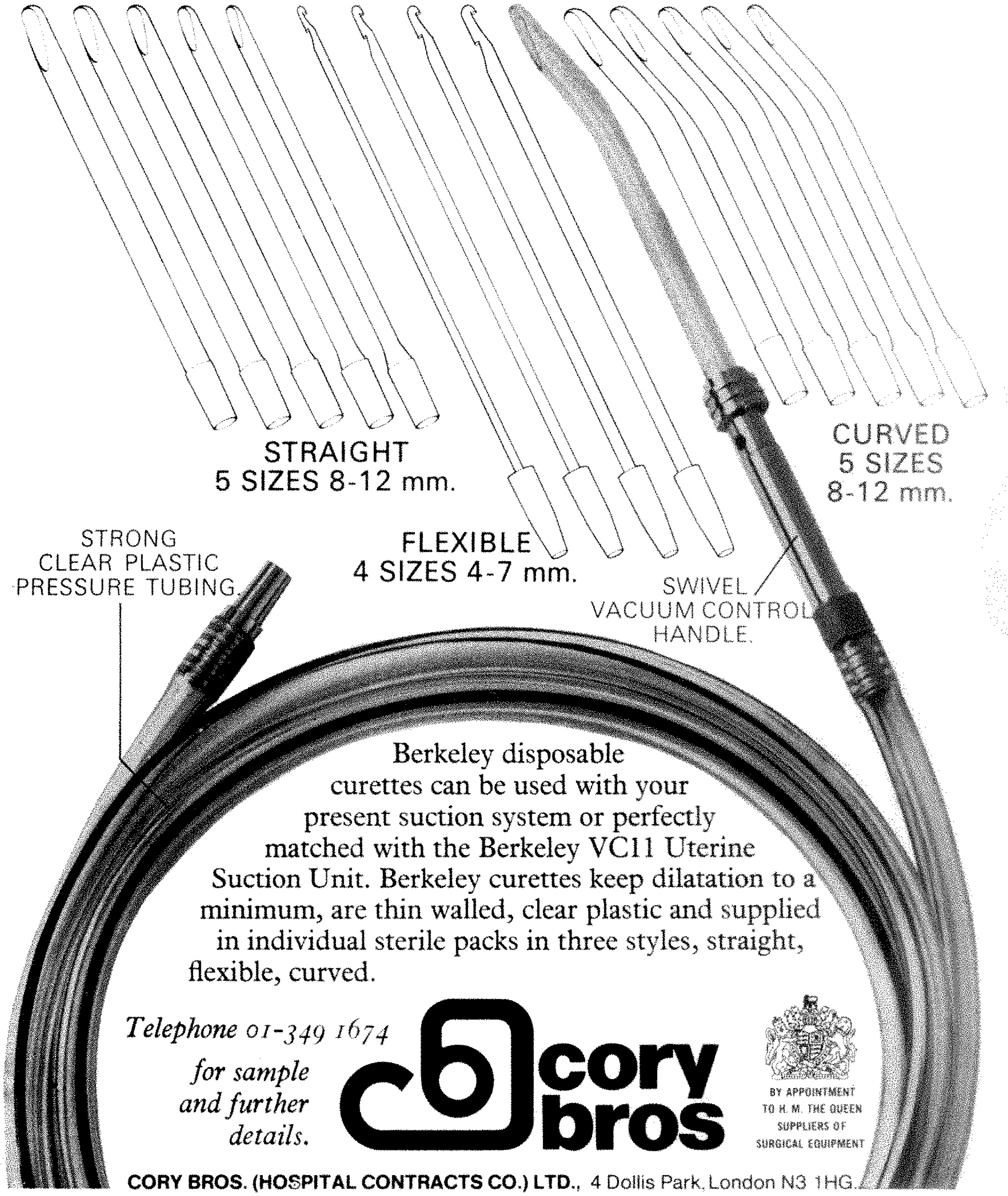

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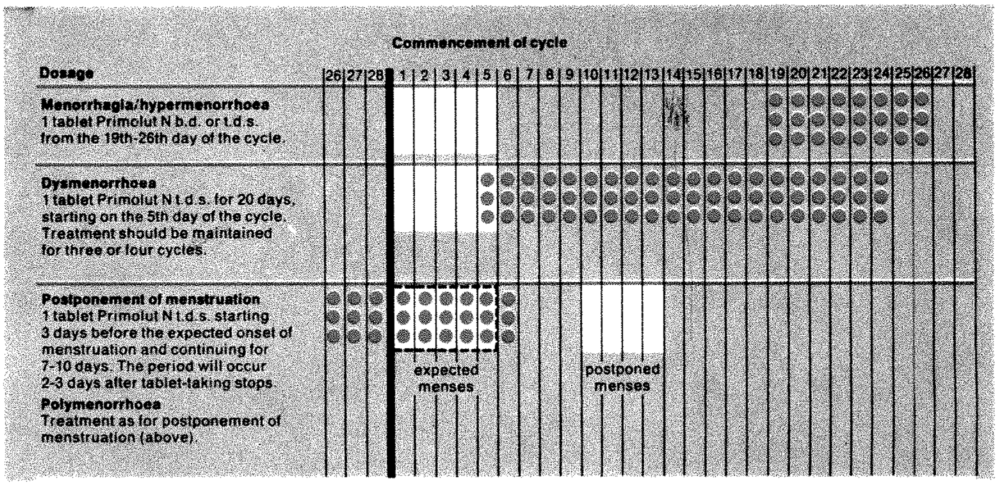

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# British Journal of Obstetrics and Gynaecology

## A CHANGE OF TITLE

As this journal enters its 74th year it takes on its third title and becomes the *British Journal of Obstetrics and Gynaecology*. In 1902 it started as *The Journal of Obstetrics and Gynaecology of the British Empire* and in 1961 *British Empire* changed to *British Commonwealth*. Since then it has become customary to speak of the "Commonwealth" rather than the "British Commonwealth" and so a further change of title seemed indicated, the choice being between *Commonwealth Journal* . . . or *British Journal of Obstetrics and Gynaecology*. Because at least four of the thirty-two Commonwealth States (namely India, Ceylon or Sri Lanka, Australia and New Zealand) were involved in specialist journals of their own it was natural for the Journal Committee and College Council to choose as title *British Journal of Obstetrics and Gynaecology*, and that is how we start 1975, a year when it is hoped gradually to introduce into our publication the revised and extended metric system known as the "Système International d'Unités (SI)".

In 1902, those producing the journal wanted it to be "a complete and impartial record of British work, an exponent of British obstetrical and gynaecological practice". This is still the aim but for some time we have also welcomed contributions from English-speaking nations outside the United Kingdom and indeed from all who like to use the English language to communicate and give the now *British Journal of Obstetrics and Gynaecology* an international flavour.

F. E. LOEFFLER

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# INFERTILITY: A COMPREHENSIVE PROGRAMME*

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SINCE 1958 the treatment of infertile couples has been transformed by ovulation induction using human follicle stimulating hormone (FSH) (Gemzell *et al.*, 1958), and clomiphene citrate (Greenblatt *et al.*, 1961; Kistner and Smith, 1961). Our South Australian experience since 1963, when we first extracted FSH from human pituitaries, is recorded here, and it follows a pattern previously reported by Townsend *et al.* (1966), which makes use of the assay techniques of Brown (1955) and Cox (1968) to monitor daily the results of treatment. In 1967 a national pituitary collection system was inaugurated by the Australian Government Department of Health, and we have used hormones supplied by it since 1969.

From 1964 to 1973, 900 couples were admitted to our clinic series of whom all but 41 (4·6 per cent) were completely investigated. Some of the latter expected a prescription for a "fertility pill" without any preliminary diagnosis, while others were unwilling to involve their partner in the investigation.

The proportions of the various infertility factors are given in Table I and are probably not representative of the overall proportions in South Australia. Because many patients were referred after full investigation by gynaecological colleagues the incidence of ovulatory failure is unusually high while the role apparently played by tubal factors is low. All patients thought to require gonadotrophin treatment were referred to us, because of the close laboratory control we could provide. The types of ovulatory failure are shown in Table II.

## OVULATION STIMULATION

When the investigation of the couple was complete, and the diagnosis of ovulatory failure had been made conclusively by hormone assay, the anovulatory woman was briefed carefully on the plan of treatment and the chances of success. We tried to admit to therapy only highly motivated women. Their management depended on monitoring ovarian function by urinary assays of oestrone and pregnanediol, so that they had to be willing to collect 24-hour samples of urine as directed, and send or bring them punctually to the hospital. Although other methods of assessing ovulation, such as basal temperature charts and plasma hormone assays were also used, we found that the patients usually preferred us to use urine assays.

In most patients, treatment began with clomiphene, the initial dose being judged by the clinical history. For those with anovulatory menses, 25 mg. daily from the fifth to ninth days of the menstrual cycle usually achieved ovulation, but higher doses were used if necessary. No time was wasted if every cycle was monitored by ovarian steroid assay; one knew if treatment was effective and if not the dose of clomiphene was increased to 50 mg., then 100 mg., and finally 150 mg. daily until regular ovulation was indicated by luteal phase values of pregnanediol greater than 2 mg. per 24 hours.

Failure to achieve ovulation with clomiphene forms an indication to use human FSH. The Human Pituitary Advisory Committee had drawn up guidelines indicating the types of patients likely to benefit from this treatment. One condition required was a failure of clomiphene. Application to the Australian Department of Health was made on special forms designed

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* Based on the Green-Armytage Anglo-American Lecture presented at the Royal College of Obstetricians and Gynaecologists, London, on 8th May, 1974.

TABLE I

*Main factors causing infertility in 900 couples referred between 1964 and 1973*

| Infertility factors | No. | Per cent |
|---|---|---|
| Ovulatory failure | 386 | 42·9 |
| Tubal obstruction | 99 | 11·0 |
| Male factors | 177 | 19·7 |
| Apparently normal couple | 158 | 17·6 |
| Congenital anomalies | 11 | 1·2 |
| Premature menopause | 4 | 0·4 |
| Treatment offered and refused | 24 | 2·7 |
| Investigations deferred or incomplete | 41 | 4·6 |

TABLE II

*Type of ovulatory failure in 386 women*

| | No. | Per cent |
|---|---|---|
| Primary amenorrhoea | 10 | 3 |
| Secondary amenorrhoea | 135 | 35 |
| Oligomenorrhoea | 165 | 43 |
| Anovulatory menses | 76 | 20 |

for computer analysis and, if approved, a supply of hormone sufficient for three average dosage cycles of treatment was sent. In each treatment cycle, information about daily dosage and the results of hormone assays had to be returned to the Department for the Committee's perusal, and for computer processing. A complete dossier of clinical conditions and results of treatment regimes including successful pregnancy was maintained. This co-operative effort on a national scale was unique, was greatly appreciated by both patients and doctors, and should produce interesting scientific data when analysed.

The first treatment cycle with FSH was really an ovarian function test (Shearman, 1964; Cox *et al.*, 1966) and consisted of three daily injections each of about 300 IU of FSH together with daily assays of oestrogen excretion for a week. The object of this was to assess the likely regular dosage necessary in subsequent cycles. In the early years of our study we used the test as a preliminary test of function before using clomiphene or FSH and the results have already been reported.

Great emphasis was placed on daily monitoring of the ovarian response during treatment with gonadotrophins. The patient brought the previous 24-hour collection of urine to the hospital before 0900 and received the prescribed injection of FSH; the assay result was available by 1700 hours so that the injection for the succeeding day could be prescribed. To avoid overstimulation, the injection of human chorionic gonadotrophin (HCG) was usually given after an interval of one day from the last FSH injection. During the luteal phase, two additional small doses of HCG (usually 500 units) were given to support the corpus luteum. Minimum dosage was used throughout (Cox *et al.*, 1968).

Combinations of therapy were sometimes used, for example, when clomiphene induced menses without ovulation. Here an injection of HCG at mid-cycle might produce ovulation, if the oestrogenic response up to that time had been sufficient. Another combination was clomiphene plus FSH. In two patients with amenorrhoea, in whom clomiphene and then large doses of FSH produced little ovarian activity, this combination of clomiphene and FSH produced ovulation, and one patient became pregnant.

The use of gonadotrophin releasing hormone may provide a useful method of therapy, but our experience is limited to a few weeks of trial.

## RESULTS

The series treated comprised 386 women, but to give a true picture of the results of treatment, 38 whose motivation extended to only one or two cycles or who had commenced treatment and were still in the first two cycles at December 1973, were excluded. Also excluded were 29 women with other infertility factors such as tubal pathology (14 patients), or husbands with less than 15 million sperms per ml. (10 patients), psychiatric imbalances (3 patients), or a premature menopause (2 patients). The remaining 319 patients had no other apparent infertility factor and the percentage figures are based on them.

Of the 319 couples remaining who had been admitted to the treatment programme after full investigation, 211 (66 per cent) eventually achieved one or more pregnancies. Ten actually conceived after investigation but before treatment could be commenced. Twenty-two others withdrew from treatment after many months but conceived due to spontaneous ovulation at a later date. The remaining 179 (56 per cent) conceived as a direct result of treatment, and 210 pregnancies occurred. One patient had four pregnancies after induced ovulation; the first two ended in spontaneous abortion. In 13 women (4 per cent) ovulation had not occurred when they withdrew from treatment after more than three months.

In those women becoming pregnant, the average number of ovular cycles up to conception was 3·4. Of all treated patients, 36·7 per cent became pregnant in the first two ovular cycles, the same proportion in the third and fourth cycles, and the same proportion of those remaining in subsequent cycles. There was therefore every reason to encourage patients to remain in the treatment programme. But some reassessment should be made every six months in case some other fertility factor has not been detected. One patient actually had 39 treatment cycles before successful pregnancy. A total of 172 pregnancies followed treatment with clomiphene (used either alone or in combination with HCG), while 38 pregnancies followed treatment with gonadotrophins, the latter being combined with clomiphene on one occasion.

Following successful pregnancy or even ovulatory stimulation without conception, 52 spontaneous ovulations with pregnancy occurred in previously treated women. Whether this was a result of treatment improving hypothalamic-pituitary function, or whether it represented a spontaneous cure rate of oligomenorrhoea and amenorrhoea is arguable. Our previous report on 136 patients (Matthews and Cox, 1973) showed that 58 per cent improved in menstrual function; 36 per cent remained unchanged, while the remaining 5 per cent got worse.

The successful survival of quintuplets and quadruplets reported (often in the lay press) is a triumph for neonatology, but not for gynaecological endocrinology. The object of ovulation induction should be to ripen fully only one follicle on each occasion. To this end we have been ultra cautious in trying to avoid overstimulation, but even so there were three sets of triplets, two of which were aborted before viability. In the clomiphene series, only twins occurred, and in every case there was a family history. The multiple pregnancy rate with FSH was low and compared very favourably with other large series reported (Table III).

TABLE III

*Multiple pregnancy due to ovulation induction*

| Type of multiple pregnancy | No. | Per cent |
|---|---|---|
| Twins | 12 out of 210 | 5·7 |
| Triplets | 3 out of 210 | 1·4 |
| Following treatment by FSH | 7 out of 38 | 18·4 |
| Following treatment by Clomiphene | 8 out of 172 | 4·7 |

Severe overstimulation, with enlargement of the ovaries to the level of the umbilicus, occurred in the first patient treated; fortunately this subsided spontaneously. Two patients with ovarian torsion early in pregnancy had mildly over-estimated ovaries which were 6 to 8 cm. in diameter.

There was no evidence to suggest that the incidence of congenital anomalies was above normal. In the series of 249 infants, one (of a set of twins) had a large exomphalos incompatible with life, the other was normal. No genetic defects were observed.

The overall abortion rate in our series was 12·5 per cent (Table IV). When conception

TABLE IV

*Incidence of abortions in various groups of patients who became pregnant as a direct result of, or at some time after, induced ovulation*

| Groups | Conceptions | Abortions | |
|---|---|---|---|
| | | No. | Per cent |
| Total pregnancies | 272 | 34 | 12·5 |
| Pregnancy after | | | |
| First treatment cycle | 38 | 5 | 13·2 |
| Clomiphene | 172 | 19 | 11·0 |
| FSH | 38 | 8 | 21·1 |
| Singleton pregnancy after FSH | 35 | 5 | 14·3 |
| Pregnancy after | | | |
| Spontaneous ovulation | 62 | 7 | 11·5 |
| Multiple pregnancy | 16 | 3 | 18·8 |

occurred in the first treatment cycle, the abortion rate was no higher, indicating that the uterus, if implantation occurred, was hormonally and structurally ready. There appeared to be a difference in abortion rates between clomiphene and FSH induced pregnancies, the respective rates being 11·0 and 21·1 per cent. Most of this difference was due to three multiple pregnancies following FSH treatment (two sets of triplets and one set of twins), which miscarried in the second trimester. The abortion rate following spontaneous ovulations was only 11·5 per cent.

### TUBAL OCCLUSION

Previous lectures in the Green-Armytage series have discussed tubal occlusion in infertility; Stallworthy (1948) emphasized the importance of defining normality, Steptoe (1970) described the use of the laparoscope, and Green-Armytage (1959) discussed operative technique.

The women in our series probably represented a harder core of tubal occlusion than in the average gynaecological practice. Reconstructive surgery was seldom offered, and only very rarely successful (Table V). The pregnancy rate of 14 per cent is not very different to that reported by Bayard Carter (1963) who claimed less than 10 per cent success.

TABLE V

*Tubal obstruction in 113 women*

| | Total No. | Pregnancy No. | Pregnancy Per cent |
|---|---|---|---|
| No operation (including 14 anovular patients) | 78 | – | – |
| Total operations | 35 | 5 | 14 |
| Tubal implantation | 10 | 2 | 20 |
| Salpingostomy | 25 | 3 | 12 |

The use of the laparoscope to detect peritubal adhesions amenable to surgery, and also to demonstrate normal tubes when other tests have been equivocal, is a most important item in infertility investigation.

### THE MALE FACTOR

Previously, interest in the male partner has been perfunctory, because no treatment produced a dramatic effect. But proper investigation is necessary and in a few cases, where spermatogenesis is faulty due to hypopituitary hypogonadal states, recovery may occur with FSH treatment. The cost is high because the sperm takes 70 days to mature, but in Australia, supplies of hormone are available for the very few men who fulfil the criteria. The incidence of azoospermia in our series may have been high because it included referrals to the sperm bank (Table VI).

TABLE VI

*Infertility factors in 189 men*

| | No. | Per cent |
|---|---|---|
| Azoospermia | 62 | 33 |
| Oligospermia | 99 | 52 |
| Poor motility (normal count) | 17 | 9 |
| Miscellaneous (infection, etc.) | 10 | 5 |

For those men who cannot be treated, the offer of donor insemination may be acceptable. A vacation project by student M. C. Low showed that a controlled rate of freezing in a suitable medium containing egg yolk, glycerol, glucose, citrate, glycine and erythromycin, allowed more than 50 per cent regeneration of living human sperms. A sperm bank and donor panel were arranged, and initial success was encouraging. A total of 39 couples with complete or virtual azoospermia in the husband were treated, and 23 have had a fair trial of three months or more: 11 pregnancies occurred, the average number of ovular cycles for each conception being 3·4. All completed pregnancies resulted in the birth of normal children. Selection and screening of donors, and matching of couples required much organization, but the bank offered more opportunity for scientific control, than did calling fresh donors as required.

The sperm bank also allowed "prophylactic" treatment of infertility. Immunosuppressant drugs affect spermatogenesis, so that the recipients of renal transplants become infertile. Preservation of semen produced before operation may allow the continuation of the family in those cases where successful physical recovery occurs.

## PSYCHOLOGICAL FACTORS

The tensions of investigation and treatment and disappointments at the lack of therapeutic success may cause psychological upset and sexual frustration. It is of no profit in managing infertility if the regime causes marital break-up.

Mai *et al.* (1972) studied 50 consecutive couples referred to the infertility clinic and a control group from antenatal and family planning clinics. Significantly more of the infertile women were diagnosed as having an hysterical or aggressive personality disorder, than were the fertile women. The infertile wives also gave several indications of disturbances in sexual orientation and behaviour. There was, however, no evidence of a greater incidence of neurosis or psychosis in the infertile group.

Although it was our practice to recommend a rest from treatment whenever tensions or anxiety built up, there were couples who needed psychiatric help. Four became quite disturbed, and one withdrew from treatment on this account. Psychological screening of women with unexplained infertility (Carter, 1963), showed a pregnancy rate of 47·6 per cent in the "inadequate psychosomatic group" compared with 57·8 per cent in the "adequate psychosomatic group".

The clinical history taken by the gynaecologist should expose those couples with psychological or sexual problems who need additional help. In a proportion of these, the help of an interested psychiatrist is most valuable.

## CONCLUSION

A comprehensive clinic service such as that described above must be a regional one, because of the sophistication of the laboratory support necessary. The results reported come from an underpopulated state extending hundreds of miles from the centre and with just over a million people. They reflect co-operation between patients and their doctors, and between gynaecologists and the comprehensive clinic when special treatment control is required. The first 89 patients in this study were the subject of a report by Black *et al.* (1969) and to date 71 of them (80 per cent) have become pregnant.

## ACKNOWLEDGEMENTS

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# ENGAGEMENT OF THE FETAL HEAD IN PRIMIGRAVIDAE AND ITS RELATIONSHIP TO DURATION OF GESTATION AND TIME OF ONSET OF LABOUR

BY

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## Summary

In a retrospective survey on the time of engagement of the fetal head in 462 unselected consecutive primigravidae it was seen that by the end of the 37th week of gestation (259 days), engagement had occurred in only 23 per cent of them. The highest rate of engagement was from 39 to 40 weeks of gestation (273 to 280 days) and in 50 per cent of patients, engagement occurred between 38 and 42 weeks (266 to 294 days). The mean interval between engagement and delivery was 1·39 weeks (9·7 days), the median was 7 days, and the mode was less than 7 days. In 80 per cent of patients the engagement-delivery interval was less than 14 days. These findings strongly suggest that in the majority of primigravid patients the fetal head does not engage between 36 and 38 weeks of gestation (252 to 266 days) as is commonly believed.

TRADITIONALLY, engagement of the fetal head is said to take place at about 36 weeks of gestation in primigravidae (Percival, 1969), being expected to occur at this time of pregnancy in some 80 per cent of women (Percival, 1969). Engagement has been defined as the passage of the biparietal diameter of the fetal skull through the plane of the pelvic inlet (Donald, 1969). In clinical terms this means that very little of the fetal head can be felt on abdominal examination and the examining fingers cannot be dipped below the presenting part (Clayton *et al.*, 1972). The recognition of this phenomenon in the antenatal clinic is, however, dependent upon clinical skill and experience. The more recent practice of describing the level of the fetal head in fifths above the pelvic brim was not in use at the time of this survey (Philpott and Castle, 1972). Most obstetricians will have encountered primigravidae in whom engagement of the fetal head has been recognized even as early as 30 weeks of gestation in a woman "sure of her dates" and will also have seen those with the phenomenon of the "high head at term". As a result of a clinical impression that engagement of the fetal head very frequently had not occurred at 38 weeks in primigravid patients, and in the absence of any recent comprehensive studies on the subject, we were prompted to investigate the frequency distribution of the duration of gestation at engagement in all primigravidae attending the antenatal clinic at one hospital over the period of 1971 to 1972, and to attempt

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to see what was the relationship between duration of gestation at first reported engagement, and duration of gestation at the time of delivery.

## METHODS

This analysis is based on a retrospective survey of the antenatal records and records of delivery of 462 consecutive primigravidae who had a labour of spontaneous onset and were delivered after 30 weeks of pregnancy at the Bearsted Memorial Hospital during 1971 and 1972. Patients were examined from 28 weeks at two-weekly intervals until 36 weeks and thereafter weekly until delivered. From the case notes was recorded the time that engagement of the fetal head with the patient in the supine position was first reported. Engagement was deemed to have taken place when the examining fingers could not be dipped below the presenting part and very little of the fetal head could be felt on abdominal examination. This corresponds with, two-fifths or less of the fetal head being above the pelvic brim according to the current method described by Philpott and Castle (Philpott and Castle, 1972). All patients were examined by one of six doctors, with at least two years' experience in clinical obstetrics. The figures were then analyzed to assess the relationship between first reported engagement of the head and duration of gestation, and duration of gestation at delivery.

Included in the study were patients who booked before 16 weeks of gestation, were sure of their dates, had regular cycles of less than 32 days and in whom the uterine size at booking was compatible with the calculated duration of gestation.

Excluded were patients who were unsure of their dates, patients who delivered at other hospitals, patients with a multiple pregnancy, patients in whom the breech presented after 32 weeks and patients whose notes contained inadequate information about engagement of the fetal head or vague descriptive terms such as "fixed" or "engaging". Forty women were thus omitted from the final analysis.

## RESULTS

### *Duration of gestation at first reported engagement*

In this series, the mean time of first reported engagement of the fetal head was 271±11·9 (SD) days of gestation (38·67±1·7 weeks), the median was 273 days (39 weeks), and the mode was 280 days (40 weeks) (Table I).

TABLE I

*Time of engagement, time of delivery, and engagement-delivery interval*

| | Mean | Standard deviation | Median | Mode |
|---|---|---|---|---|
| Duration of gestation at first recorded engagement (days) | 271 | 11·9 | 273 | 280 |
| Duration of gestation at time of delivery (days) | 278·6 | 10·3 | 280 | 280 |
| Engagement-delivery interval (days) | 9·7 | 13·3 | 7 | Less than 7 |

Figure 1 shows the cumulative incidence of engagement between 210 and 294 days (30 to 42 weeks of gestation); 24 per cent (101 patients) of the studied population were coloured. Figure 1 shows that this fraction of the population studied did not significantly differ from the total population with respect to time of first reported engagement. Fifty per cent of the patients were seen to have an engaged head by 266 days of gestation (the beginning of the 39th week), but at 273 days of gestation (the end of the 39th week), 35 per cent of the patients were still not reported to have an engaged head. At 259 days of gestation (37 weeks), the time when the fetal head is clinically expected to engage in the primigravid patient, engagement was found in only 23 per cent of all the patients.

Figure 2 indicates the frequency distribution of the time of the first reported engagement of the fetal head. The greatest incidence of the finding of engagement for the first time occurred at 280 days of gestation (40 weeks). The highest rate of engagement occurred between 273 and 280 days of gestation (39 and 40 weeks). In 50 per cent of primigravid patients studied, engagement occurred between 266 to 294 days of gestation (38 to 42 weeks).

### *Relationship between time of first engagement and time of delivery*

Figure 3 shows the cumulative incidence of delivery in the studied population. The mean

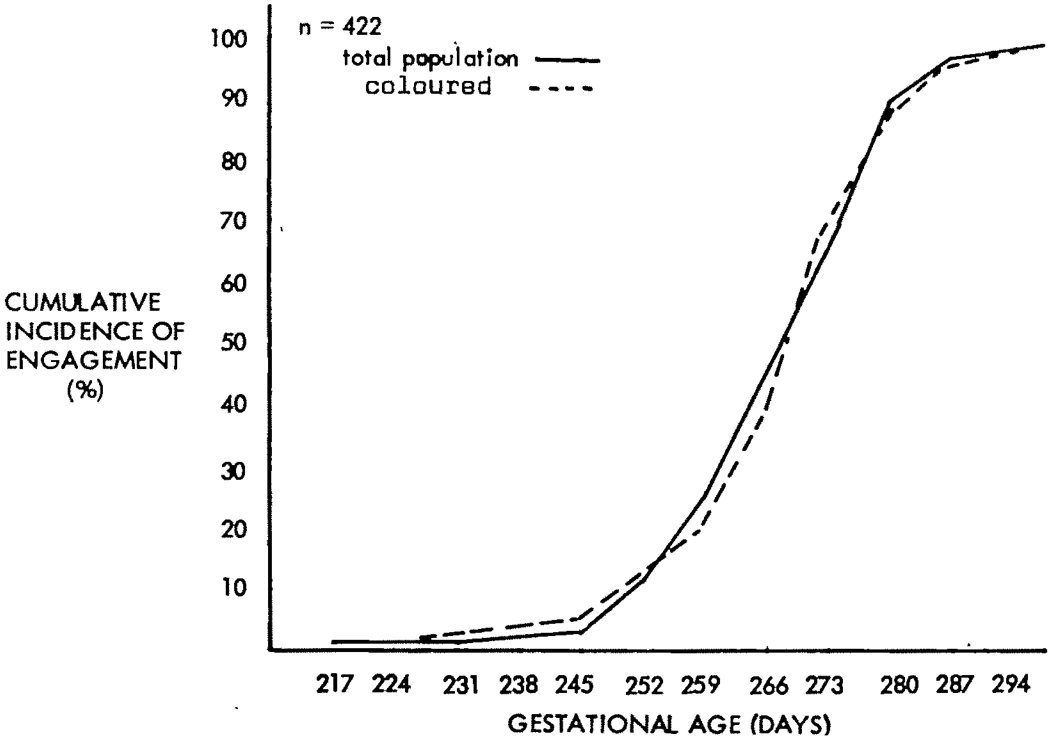

FIG. 1
Cumulative incidence of first reported engagement of the fetal head in primigravidae.

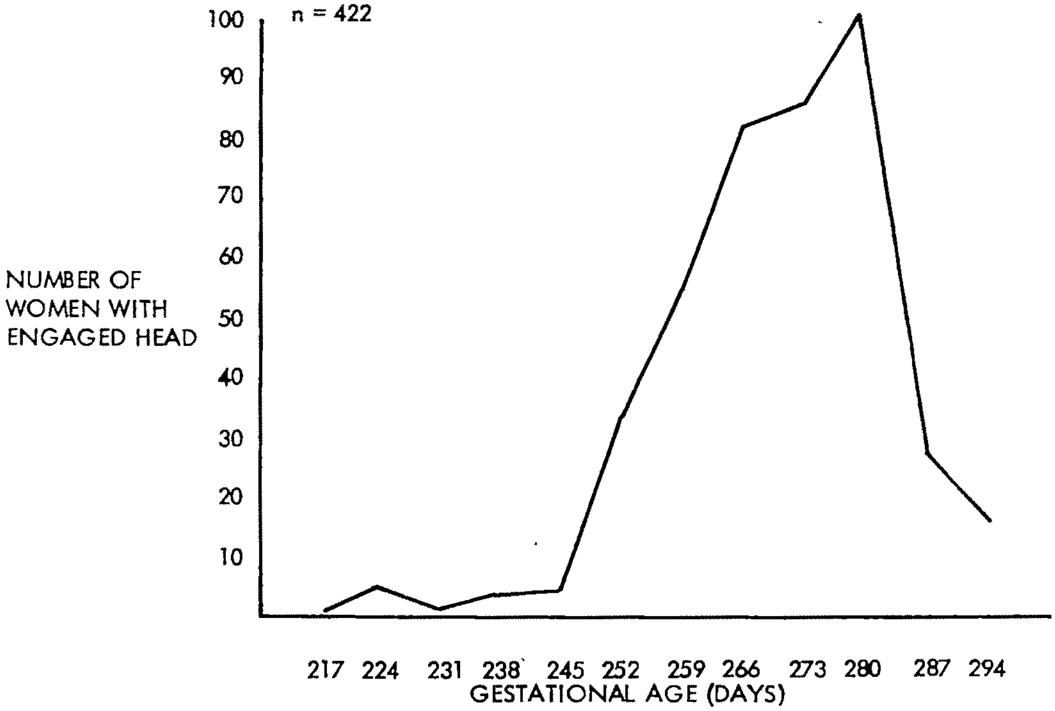

FIG. 2
Frequency distribution of the time of first reported engagement of the fetal head in primigravidae.

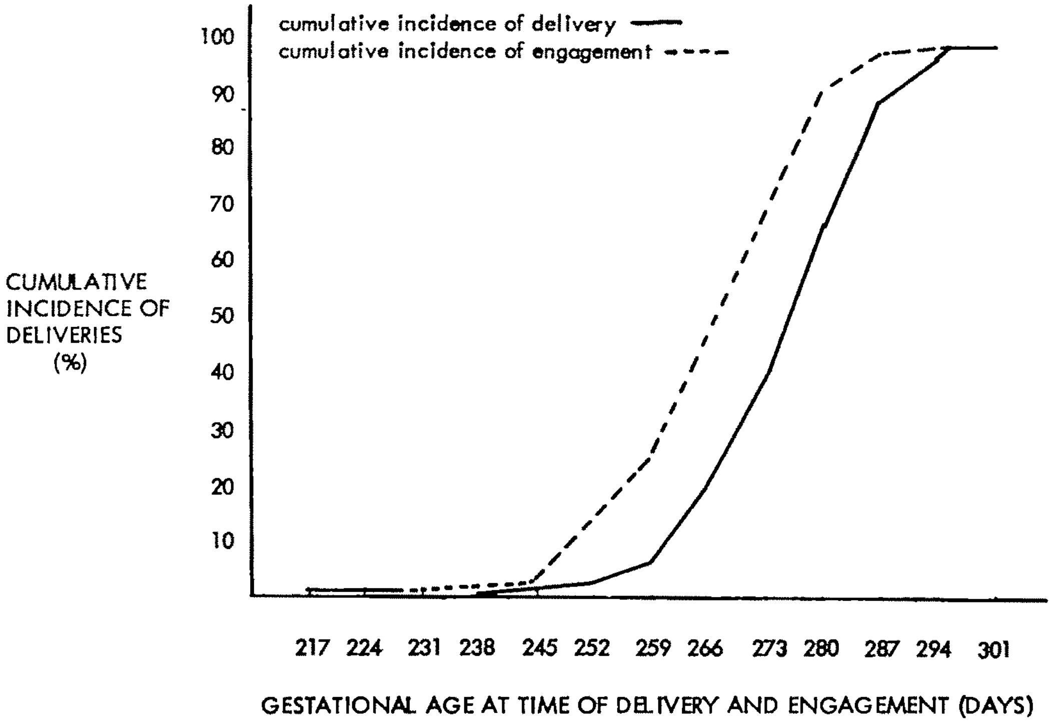

FIG. 3
Cumulative incidence of engagement of the fetal head and delivery in primigravidae.

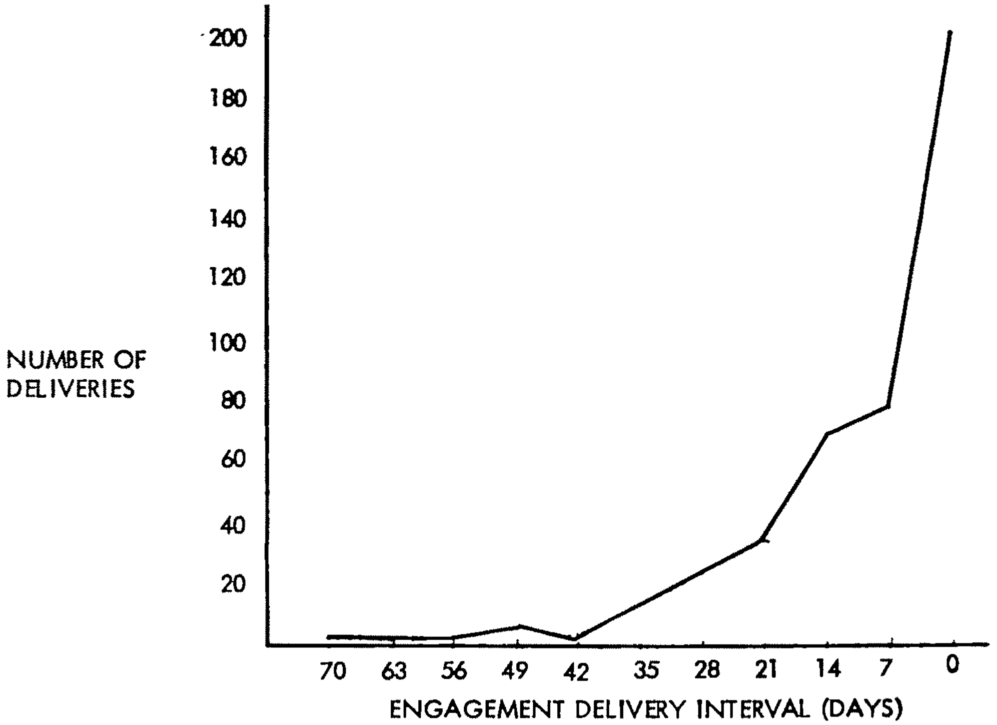

FIG. 4
Engagement delivery intervals in primigravidae.

gestational age at delivery was 279±10·5 (SD) days (39·8±1·48 weeks (and the median and mode were 280 days (40 weeks). The mean interval between the first reported engagement and delivery was 10±13·3 (SD) days (1·39±1·94 weeks), the median was 7 days, and the mode less than 7 days (see Figs. 3 and 4). In 44 per cent of the patients studied, the head was not reported to be engaged until they presented in labour.

In only 20 per cent of patients was the engagement-delivery interval more than 14 days. In only 7 of the 422 patients included in the survey was an unengaged head at term associated with cephalopelvic disproportion or placenta praevia.

## COMMENT

Many standard obstetric textbooks state that spontaneous engagement of the fetal head in primigravidae should have occurred by the 37th week of pregnancy and suggest that failure to do so is an indication for further investigations. The results of this survey are not in agreement with this teaching. They suggest that only a minority of primigravidae will have an engaged fetal head in the supine position by the end of the 37th week (259 days), and in the majority of patients engagement takes place after the end of the 38th week of pregnancy (266 days). There was a close relationship between engagement of the fetal head and duration of gestation at delivery. Eighty per cent of patients in this series delivered within 14 days of the first reported engagement and 46 per cent within 7 days. Nevertheless, in 5 per cent of patients the engagement delivery interval was 6 weeks (42 days) or more. The observation that in 80 per cent of patients delivery occurred with 14 days of engagement could, in conjunction with other facts, be of limited value for predicting the expected date of delivery. Failure of engagement of the fetal head up to the end of the 38th week of pregnancy (266 days) seemed to be a normal occurrence in the population studied; only occasionally was underlying obstetric pathology present.

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# TRANSFER OF FOLATE TO THE FETUS

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## Summary

The accumulation of tritium-labelled folate in the product of conception was examined in eight women undergoing hysterotomy for termination of pregnancy. Folic acid containing a small quantity of tracer material (50n Ci/$\mu$g.) was injected intravenously 20 minutes before operation in 4 patients but 24 hours beforehand in the remainder. After operation, maternal blood, placenta, fetal blood, and fetal liver were examined for radioactivity as was the daily urine output during the patient's postoperative hospital stay. The results indicated that only ½ per cent of the injected material accumulated in the product of conception—uptake was primarily by the fetal liver and no large placental pool of folate was involved. Approximately half of the injected load was excreted in the urine—the majority in the first 24 hours—and the remainder was presumed to have been retained by maternal tissues. Bioassay with *L. casei* and chromatography on TEAE cellulose suggested that extensive metabolism of the injected material had taken place and that fetal uptake might be more efficient than indicated by counting radioactivity alone.

It has been established that cord blood folate levels are generally above the adult mean (Vanier and Tyas, 1966; Roberts *et al.*, 1969; Landon and Oxley, 1971), and it is generally accepted that the transfer of folate from the mother to the fetus, to maintain this differential is in part responsible for some degree of maternal folate deficiency which is manifest by low blood folate levels, increased clearance of an intravenous load and occasionally by megaloblastic anaemia (Chanarin *et al.*, 1959; Chanarin, 1969; Hansen and Klewesahl-Palm, 1963).

Although the fetus has a reputation for avidly acquiring folate and other nutrients at the expense of its mother, nothing is known about how this is achieved, or how the fetus responds to changes in its maternal supply. By artificially raising maternal levels both vitamin $B_{12}$ and iron have been shown to move from the maternal circulation very rapidly—vitamin $B_{12}$ tending to accumulate in the placenta while iron passes rapidly to the fetus (Luhby *et al.*, 1958; Fletcher and Suter, 1969). Similar work on rats suggests that some 25 per cent of an injected load of folic acid might accumulate in the placenta within 30 minutes (Copenhauer and O'Brien, 1969).

The present investigation was undertaken to see whether similar placental uptake was characteristic of human pregnancy.

## Patients and Methods

### *Patients*

Ideally, healthy young women undergoing termination of pregnancy at a uniform stage of pregnancy would have been selected. In the event, the availability of labelled folic acid, which had to be used within a few weeks of its preparation dictated the time of the experiments and the ideal could not be met. Relevant clinical details of the patients are given in Table I. All had a termination of pregnancy for "social" reasons except subject C who had multiple sclerosis.

### *Methods*

Tritium labelled folic acid (TRK.212 [3′5′9(n)–$^{3}$H] folic acid, K salt was purchased from the Radiochemical Centre, Amersham. Only freshly prepared material was used. On receipt, the radioactive material was diluted with non-radioactive folic acid (U.S.P.) to give a working solution containing 1 mg./ml. folic acid with a specific activity of 50 μCi/mg. This was stored at —196 °C. in liquid nitrogen while an aliquot was tested at the Huntingdon Research Centre for sterility and apyrogenicity. Chromatography of two further aliquots on TEAE cellulose revealed no significant chemical or microbiological impurity.

Every patient received an intravenous injection of 1·5 mg. folic acid labelled with 75 μCi of tritiated material. The labelled folate was administered within 30 *minutes* of operation in four cases and approximately 24 *hours* beforehand in the remaining four cases.

A maternal blood sample was taken at the time of delivery, after which the product of conception was weighed, rapidly dissected and the appropriate tissues stored at —20 °C. An attempt was made to collect all the urine passed by these patients during their postoperative stay in hospital and on the day before discharge an oral dose of 30 mg. of folic acid was given in an attempt to "flush out" labelled folate from maternal tissues.

An accurately weighed aliquot of homogenized tissue (about 50 mg.) was heated for 60 minutes at 70 °C. with 0·2 ml. of 5N. NaOH in a counting vial. Distilled water (0·2 ml.) was then added followed by BBS 2 solubilizer (Beckman Instrument Co. Ltd.) until the reaction mixture was neutral. Plasma and urine samples were solubilized with BBS 3 without any previous preparation. A toluene based scintillant was used (10 ml./vial) and the samples counted until the standard deviation of their net counts was 1 per cent or less; efficiencies ranged from 23 to 37 per cent.

Samples of placenta and fetal liver were extracted in 0·1M potassium phosphate buffer pH 8·0 containing 1 per cent ascorbic acid (Bennett *et al.*, 1964) and aliquots analysed by chromatography on 10 × 100 mm. columns of TEAE cellulose (Cellex T-Calbiochem) following the procedures outlined by Usdin (1959) and Schertel *et al.* (1965). Samples of plasma and urine were also analysed but without previous extraction. The column eluate was collected in 4 ml. fractions and mixed directly with 10 ml. Triton X—100/toluene scintillant for counting (Turner, 1969). Using this system the baseline counting rate in chromatograms of extracts of fetal liver and of placentae was invariably

Table I

*Subjects studied*

| Subject | Age (years) | Height (cm.) | Weight (kg.) | Parity | Gestation (weeks) | Birth weight of fetus (g.) |
|---|---|---|---|---|---|---|
| A | 41 | 168 | 82·8 | nd | 13 | 41·2 |
| B | 37 | nd | 42·8 | 5 | 12 | 153·0 |
| C | 43 | 161 | 63·0 | 4 | 18 | 72·0 |
| D | 32 | 165 | 95·2 | 2 | 13 | 32·5 |
| E | 42 | nd | nd | 7 | 18 | 223·0 |
| F | 28 | nd | nd | 3+1 | 14 | 78 2 |
| G | 24 | 180 | 57·8 | 2 | 17 | 103 5 |
| H | 26 | 159 | 70·1 | 5 | 14 | 68·2 |

higher than in those of plasma and urine samples.

Total folate activity of extracts, plasma and urine was estimated microbiologically using a direct addition technique (Herbert, 1966) with *L. casei* NCIB 8010 as test organism.

## RESULTS

Following the intravenous load there was a rapid disappearance of the injected material from the maternal circulation so that after 20 minutes only some 15 per cent remained (Table II). After 24 hours less than 1 per cent remained (Table III).

After 20 minutes the uterine contents had accumulated about 0·1 per cent of the injected label; the majority was found in the placenta. In concentration the fetal liver was one-quarter of that of the placenta (Table II). After 24 hours the placenta and fetal plasma had roughly doubled their tritium concentration while that of the fetal liver had increased over thirty-fold. Even so the total uptake was small compared with the quantity excreted by the mother during the same period.

Although urine collections were not always complete and the initial specimens were contaminated with blood, it is clear that the majority of the urinary loss occurred in the first 24 hours (Table IV). Excretion of the label continued at a small but consistent level throughout the period studied. A small increase in tritium excretion was provoked by oral administration of unlabelled folic acid, but despite this over 50 per cent of the injected dose was unaccounted for at the end of the experiment.

The quantity of tritium measured in the various samples may be expressed as "nanogrammes folate" by appropriate reference to the specific activity of the injected material. The calculated value can then be compared with that measured by *L. casei* (Table V).

In general the chromatographic analysis of

TABLE II

*Tritium accumulated after 20 minutes*

| | Patient | Time (*minutes*) | Gestation (weeks) | Mass (g.) | Percentage of injected load |
|---|---|---|---|---|---|
| Maternal Plasma | A | 9 | 13 | Assumed 3000 ml. | 16 |
| | B | 22 | 18 | | 19 |
| | C | 19 | 12 | | 13 |
| | D | 30 | 13 | | – |
| | Mean | 20 | 14 | | |
| Placenta | A | | | 50·48 | 0·088 |
| | B | | | 97·00 | 0·266 |
| | C | | | 63·00 | 0·097 |
| | D | | | 57·86 | 0·086 |
| | Mean | | | 67·08 | 0·134 |
| Fetal Plasma | A | | | Assumed 10 ml. | 0·0004 |
| | B | | | | 0·0039 |
| | C | | | | 0·0058 |
| | D | | | | 0·0079 |
| | Mean | | | | 0·0045 |
| Fetal Liver | A | | | 1·516 | 0·0005 |
| | B | | | 7·300 | 0·0056 |
| | C | | | 4·196 | 0·0016 |
| | D | | | 1·260 | 0·0007 |
| | Mean | | | 3·568 | 0·0021 |

material from these patients produced an elution pattern with three main peaks which were tentatively identified by reference to the behaviour of authentic standard compounds in the same system. The last peak of activity eluting between 160 and 240 ml. was in all general respects identical to the injected material, namely, folic acid. An area of activity eluting rather more rapidly (40 to 100 ml.) and usually consisting of two small peaks could be either

TABLE III

*Tritium accumulated after 24 hours*

| | Patient | Time (*hours*) | Gestation (weeks) | Mass (g.) | Percentage of injected load |
|---|---|---|---|---|---|
| | E | 22½ | 18 | | 0·8 |
| Maternal | F | 23 | 14 | Assumed | 1·2 |
| Plasma | G | 21¼ | 17 | 3000 ml. | 1·1 |
| | H | 25½ | 14 | | 0·7 |
| | Mean | | 16 | | 1·0 |
| | E | | | 166·26 | 0·86 |
| Placenta | F | | | 68·97 | 0 46 |
| | G | | | 86·50 | 0·26 |
| | H | | | 64·88 | 0·21 |
| | Mean | | | 96·65 | 0·45 |
| | E | | | Assumed | 0·0078 |
| Fetal | F | | | 10 ml. | 0 0086 |
| Plasma | G | | | | 0·0062 |
| | H | | | | 0·0062 |
| | Mean | | | | 0·0072 |
| | E | | | 19·210 | 0·250 |
| Fetal | F | | | 6·410 | 0·084 |
| Liver | G | | | 4·198 | 0 058 |
| | H | | | 2·567 | 0·040 |
| | Mean | | | 8·096 | 0·108 |

TABLE IV

*Tritium excretion, percentage of injected load per 24 hours*

*Values obtained after oral "flushing" dose are in italics*

| Day | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| 1 | 13·8 | 14·6 | 21·0 | 4·9 | 33·5 | 50·4 | 52·6 | 18·0 |
| 2 | 10·8 | 30·3 | 0·6 | 2·7 | 0·3 | 0·5 | 0·6 | 0·4 |
| 3 | | 0·6 | 0·3 | 0·1 | 0·5 | 0·6 | 0·5 | 0·3 |
| 4 | 0·5 | 0·4 | 0·7 | 0·3 | 0·3 | 0·4 | 0·5 | 1·2 |
| 5 | 0·3 | *5·6* | 0·6 | 0·3 | 0·6 | 0·4 | ns | 0·4 |
| 6 | 0·3 | | 0·5 | 0·2 | 0·3 | 0·4 | 0·3 | 0·3 |
| 7 | *13·4* | | 0·4 | *4·1* | 0·4 | 0·5 | 0·2 | 0·2 |
| 8 | *0·7* | | 0·3 | | *1·1* | *7·0* | 0·3 | *4·0* |
| 9 | | | 0·1 | | *0·4* | *0·6* | 0·3 | |
| 10 | | | 0·3 | | | | *3·9* | |
| 11 | | | *3·6* | | | | | |
| Total | 39·8 | 51·5 | 28·4 | 12·6 | 37·4 | 60·8 | 59·2 | 24·8 |

TABLE V

*Concentration of folate in ng./ml. or ng./g. determined by* (a) *microbiological assay* (*L. casei*) *in italics and* (b) *by tritium counting and based on specific activity of injected material*

| | A | B | C | D | Mean | E | F | G | H | Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| Maternal Plasma | 350<br>*104* | 350<br>*122* | 200<br>*88* | 375<br>*40* | 320<br>*88* | 10<br>*5* | 10<br>*8* | 9<br>*7* | 15<br>*4* | 11<br>*6* |
| Placenta | 860<br>*34* | 1080<br>*52* | 760<br>*31* | 720<br>*30* | 855<br>*37* | 620<br>*88* | 800<br>*130* | 1000<br>*56* | 480<br>*54* | 725<br>*82* |
| Fetal Plasma | 190<br>*1* | 73<br>*8* | 12<br>*12* | 24<br>*2* | 75<br>*6* | 50<br>*14* | 27<br>*16* | 26<br>*12* | 15<br>*10* | 30<br>*13* |
| Fetal Liver | 8720<br>*6* | 4880<br>*14* | 5400<br>*7* | 4532<br>*10* | 5880<br>*9* | 5000<br>*223* | 6200<br>*250* | 5600<br>*272* | 3000<br>*260* | 4950<br>*250* |

5-methyl folate or 10-formyl folate or indeed a mixture of both. It is difficult with the present system to resolve a mixture of these two derivatives without differential microbiological assay. Fractionation of normal plasma samples and analysis with *L. casei* invariably gives maximum activity in this region and it seems reasonable to assume that radioactivity eluting in this area represents labelled folate metabolized from the injected parent compound. Activity eluting in the void volume could be either protein bound folate—unlikely in urine and tissue extract—or more likely a breakdown product such as *p*-aminobenzoic acid. It was always present whatever the material examined but its significance is a matter for conjecture.

Chromatographic analysis of maternal plasma revealed that labelled derivatives appeared within 20 minutes of injecting the intravenous load and by 24 hours no folic acid itself was detectable at all. There was no evidence that the injected folic acid had accumulated within placental or fetal tissue. After 20 minutes exposure a trace of folic acid was detectable in an extract of placenta but after 24 hours this was no longer apparent. No folic acid was found in the fetal plasma or the fetal liver at any time, only labelled derivatives were detected (Fig. 1).

The majority of radioactivity excreted in the first 24 hours following the intravenous load was attributable to folic acid itself but the small amounts excreted thereafter were entirely labelled derivatives until the 24 hours following the oral "flushing" load when folic acid itself could again be detected (Fig. 2).

## DISCUSSION

The rapid disappearance of tritium labelled folic acid from the maternal circulation is in general agreement with earlier reports on plasma levels following an intravenous load. Since it is unlikely that glomerular filtration alone could account for the clearance, this very rapid fall must in part be due to distribution throughout the extracellular water and to uptake by tissue cells. The initial (20 minutes) clearance rate suggests that the maternal plasma would have fallen to approximately 1 per cent after about an hour. The persistence of the 1 per cent at least for 24 hours may represent either breakdown of the label, or leakage from the tissues to make good the renal losses or, as indicated by chromatographic analysis, the appearance of a labelled folate derivative in the peripheral circulation. The fact that over three times as much is excreted in 24 hours as is circulating at 20 minutes must imply drainage from maternal tissues. If, for example, the injected load rapidly equilibrated in 12·5 litres of extracellular water instead of 3 litres of plasma, then the urinary excretion over the first week could be attributed in part to the draining of this space. The flushing out of folic acid over a week after injection implies that not all the injected load was metabolized. Johns *et al.* (1961) reported similar results which were interpreted as indicating that folic acid was rapidly removed from the circulation and accumulated by the cells as such but then slowly converted to a non-displacable form. It is difficult to reconcile this interpretation with the present results where labelled derivatives

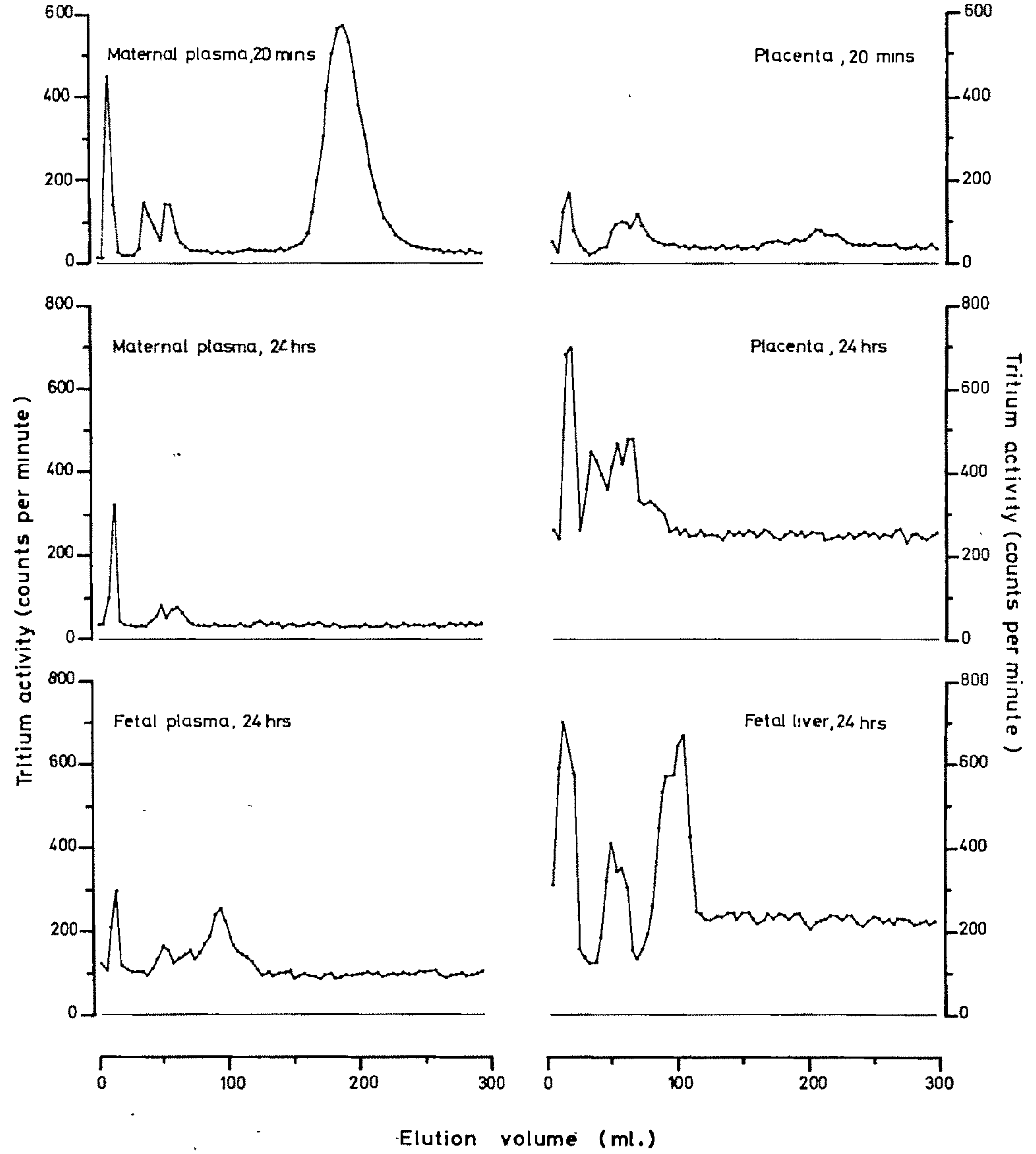

FIG. 1

Elution patterns following TEAE chromatography of samples of cord plasma (1 ml.), maternal plasma (2 ml.), placenta (≡ 5 g.), and fetal liver (≡ 1 g.) from various subjects studied.

appeared in the peripheral plasma within 20 minutes.

With the data at present available it is impossible to construct an accurate picture of folate flux across the placenta but some approximations may be made. If the rate of uptake by the fetus and placenta over 24 hours was equal to that over the first 20 minutes then the product

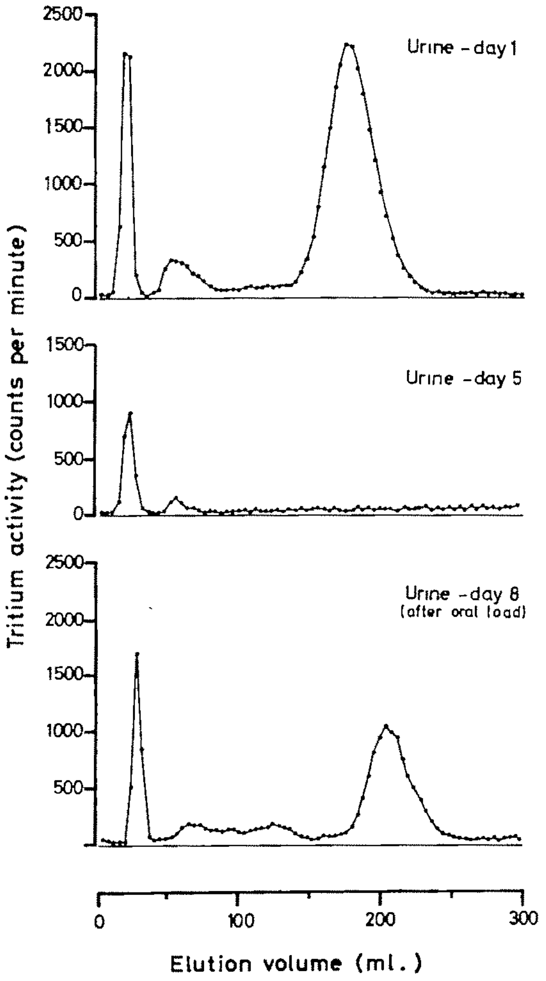

FIG. 2

Elution patterns following TEAE chromatography of urine samples from patient F. The oral load consisted of 30 mg. unlabelled folic acid 24 hours previously. A 5 ml. sample from a 24 hour collection was analysed in each case.

of conception should accumulate some 15 per cent of the injected load. This is nearly equal to the total label in the maternal circulation at 20 minutes, even if this were all available to the fetus which is unlikely. The maternal levels will continue to fall (at least to 1 per cent) and the placenta will have to compete for its supplies not only with maternal tissues but also with renal losses. If it is assumed that the maternal plasma level declines at a steady rate and the placenta (on behalf of the fetus) removes a constant proportion, then the total accumulation over 24 hours would be less than half the observed uptake.

If, however, there is a maximum rate of transfer of folate across the placenta then one of the limiting factors will be the concentration of endogenous folate, not the concentration of injected $^3$H folic acid. It is immediately apparent from Table IV that at 20 minutes folate measured by *L. casei* is considerably in excess of that calculated from tritium counting. The most likely explanation is that the injected label has displaced unlabelled folate from maternal tissues (Herbert and Zalusky, 1962; Chanarin and McLean, 1967). Although this will affect calculations at 20 minutes, it will have an even greater effect over 24 hours. Because of this displacement the specific activity in the circulating plasma is progressively reduced and at 24 hours tritium counting is likely to underestimate the total quantity of folate transferred. TEAE cellulose chromatography indicated that none of the injected *folic acid* found its way to either the placenta or the fetal liver over 24 hours as folic acid itself. The label was attached to other folate derivatives and presumably indicated rapid and extensive metabolism.

The virtual absence of folic acid itself from the placenta was surprising. Results with animal experiments suggest that metabolism of intravenous folic acid is not so rapid that none is detectable after only 20 minutes (Corrocher *et al.*, 1972; Brown *et al.*, 1974). The placenta contains appreciable levels of dihydrofolate reductase (Jarabak and Bachur, 1971), but there is no way of telling whether the labelled folates in the placenta have been metabolized *in situ* or merely taken up from those circulating in the maternal plasma. Comparison with maternal liver is impossible but it would be of great interest to know whether these two organs differed in their ability to handle folic acid.

Compared with the amount excreted and the amount presumed to have been retained by the mother, the uptake by the fetus is trivial. At best the results can only give two "stills" from a highly dynamic process—it is not possible for example to quantitate the "reverse" transfer of folate from fetus to mother. The tentative conclusion, however, is that folate uptake is primarily the concern of the fetal liver and that no intermediary storage by the placenta is involved. The concentration of *L. casei* active material in the placentae of the present series is similar to that found in placenta collected without prior loading with intravenous folic acid (Landon and Hytten, unpublished) and it seems unlikely that the intravenous load has significantly affected the total folate content of the present samples. The high levels of endogenous folate encountered in the placenta must therefore be concerned with the proper functioning of that organ. It would be of great interest to know whether placental folate can be mobilized for the benefit of the fetus in the event of folate deprivation or whether under these circumstances the placenta would compete more effectively with maternal tissue. Certainly, at this stage of gestation the results are not in keeping with the hypothesis of high fetal demand depleting limited maternal resources and the retentive capacity of the fetal liver at this stage is difficult to reconcile with the rapid fall in neonatal folate levels after delivery.

## APPENDIX

*Radiation dose*

(*a*) Maternal: assuming that on average 50 μCi remains in the maternal liver (1000 g.) and that this is excreted at a steady 0·5 μCi/day.

Total dose = 2·2 rads.

(*b*) Fetus: assuming that the fetus accumulates in its liver (10 g.):

0·084 μCi (observed)
0·31 μCi (from placenta)
0·73 μCi (via placenta from maternal plasma)

and that the rate of loss is the same as the adult.

Total dose = 5 rads.

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# FOLIC ACID ABSORPTION IN PREGNANCY

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## Summary

Folic acid absorption was studied in six non-pregnant women, and nine pregnant women undergoing therapeutic termination of pregnancy between 10 to 26 weeks, using 2 mg. of folic acid labelled with 10 μCi of tritiated folic acid. Absorption varied between 65 and 95 per cent, with a mean absorption of 80 per cent, in both pregnant and non-pregnant subjects. Similarly, there was no difference of retention between the groups. The results indicate that there is no change in the absorption of folic acid in the first 26 to 28 weeks of pregnancy.

KNOWLEDGE about the intestinal absorption of many nutrients during pregnancy is limited. While there are reports which show that pregnancy is associated with increased absorption of some minerals like iron and calcium, similar data with respect to vitamins are scanty. Available information regarding absorption of folic acid during pregnancy is conflicting. While Chanarin *et al.* (1958), Girdwood and Delamore (1961), and Giles (1966) have reported impaired absorption of folic acid during pregnancy, a recent study by Landon and Hytten (1972) found no evidence of a change. In all those studies, however, the rise in plasma folate levels after administering an oral dose of crystalline folic acid was used as an indirect measure of folate absorption in some cases after saturating the women with high doses of parenteral or oral folic acid.

Studies using radioisotopes can provide direct evidence of absorption of nutrients from the gastrointestinal tract, but the use of such compounds in pregnancy may be unjustifiable in view of possible radiation hazards to the developing fetus. From that point of view women who come for medical termination of pregnancy are more suitable subjects for study. The results of such a study using tritiated folic acid are presented.

## METHODS

Nine pregnant women between 10 to 26 weeks gestation undergoing therapeutic termination of pregnancy and six non-pregnant, non-lactating subjects undergoing sterilization were investigated. The subjects were admitted to a metabolic ward and a sample of blood collected to assess their haematological and folate status. After overnight fasting, each subject was given by mouth 10 μCi of tritiated folic acid (specific activity 1·2 Ci/mmol.) (The Radiochemical Centre, Amersham, England) mixed with 2 mg. of crystalline folic acid in 1 g. of glucose solution. The tritiated folic acid was 95 per cent pure as judged by TLC and was used within two months of delivery. None of the subjects was saturated with folic acid, but after the collection of urine and faeces the women were given flushing doses of folic acid (30 mg.) parenterally on each of three consecutive days.

Preliminary experiments had shown that almost all excretion in urine takes place in the first 48 hours, and in faeces in the first 72 hours after the dose so, to allow a margin, urine was collected for 72 hours after the dose in bottles containing toluene and mercaptoethanol as preservative and faecal samples were collected, without markers, for four days in polythene containers.

Serum and whole blood folate concentrations were determined by microbiological assay using *L. casei* as the test organism (Waters and Mollin, 1961). Haemoglobin was estimated as oxyhaemoglobin and packed cell volume (PCV) by microhaematocrit. Red blood cell (RBC) folate was calculated from whole blood folate and PCV.

Faecal samples were homogenized and radioactivity estimated by the method used by Anderson *et al.* (1960) after oxidizing an aliquot with a mixture of nitric and perchloric acids. The radioactivity was counted in aliquots taken in toluene-based Bray's mixture and counted in a Packard Tricarb liquid scintillation spectrometer model 3101. A tritium-labelled folate standard added to faeces was treated in the same way.

Urine samples were assayed for radioactivity by liquid scintillation counting by the method used by Halsted *et al.* (1967).

The values for urinary and faecal folate were expressed as percentage recovery of radioactivity as tritium. In a few samples of placenta, fetus and amniotic fluid, radioactivity was counted and was found to be less than 1 per cent of the total activity.

## RESULTS

The data are given in Table I. No significant differences in the parameters measured were observed between pregnant and non-pregnant patients.

A close correlation ($P<0{\cdot}001$; $r = 0{\cdot}86$) was observed between RBC folate levels and the percentage absorption of folic acid (Figure 1).

## DISCUSSION

Earlier observations on folic acid absorption in pregnancy have all been based on the rise in plasma folate levels following the oral administration of a known amount of folic acid. Many factors can influence the rise in plasma folic acid following ingestion of folic acid. As suggested by Landon and Hytten (1972), some may be reduced and methylated in the gut wall, some may be taken up by the liver, thus displacing 5-methyl-tetrahydrofolate into the general circulation and some may be removed from the circulation to meet tissue requirements. In addition, the volume of extracellular fluid in which the absorbed folic acid will be distributed, is considerably increased in pregnancy and wide variations are known to exist between individuals in the change in extracellular fluid volume. An increase in plasma levels of folic acid is, therefore, not a satisfactory indicator of absorption.

Data obtained here using tritiated folic acid indicates that the absorption of folate in the first 28 weeks of pregnancy is not different from that in non-pregnant subjects. Similarly, the percentage of folate retained in the tissue did not differ between the groups. Our range of figures for absorption (65 to 95 per cent) is similar to that observed by Anderson *et al.* (1960) using tritiated folic acid in adult males. The urinary excretion we observed (3 to 31 per cent) was similar to values found by Anderson *et al.* (1960). These results are in accord with those reported by Landon and Hytten (1972), who used the rise in plasma folate levels as the criterion, and found that there was no obvious change in the absorption of folate in pregnancy.

A close correlation was seen between the red cell folate levels and the percentage absorption of folic acid in the small sample studied. This observation is similar to that made in relation to iron absorption (Apte and Iyengar, 1970), where with decreasing transferrin saturation there was an increase in absorption of iron. However, the sample size is small and confirmation with larger numbers is needed.

While these data show that the absorption of folic acid is not altered during the first 26 weeks of pregnancy, they cannot be taken as indicating what may happen in the last trimester. More importantly, it is necessary to determine whether during pregnancy, absorption of the various folate derivatives in foods are in any way altered. A recent investigation of the absorption of folic acid polyglutamates (Streiff, 1970; Necheles and Snyder, 1970) in women taking oral contraceptive pills indicated that while the

TABLE I
*Folic acid absorption in pregnant and non-pregnant women*

| No. | | Haemoglobin (g./100 ml.) | PCV (per cent) | Radioactivity Folate (ng./ml.) Plasma | RBC | Recovered (per cent) Urine | Faeces | Absorption (per cent) | Retention (per cent) |
|---|---|---|---|---|---|---|---|---|---|
| | *Non-pregnant* | | | | | | | | |
| 1 | – | 14·6 | 41·0 | 8·3 | 217·0 | 18·7 | 12·3 | 87·7 | 69·0 |
| 2 | – | 11·0 | 30·0 | 5·0 | 307·0 | 3·7 | 23·6 | 76·4 | 72·7 |
| 3 | – | 14·4 | 42·0 | 7·2 | 216·0 | 20·6 | 25·1 | 74·8 | 54·2 |
| 4 | – | 13·5 | 43·0 | 8·6 | 225·0 | 18·8 | 23·6 | 76·8 | 58·7 |
| 5 | – | 11·4 | 34·0 | 8·0 | 90·0 | 29·6 | 10·6 | 89·4 | 59·8 |
| 6 | – | 11·6 | 34·0 | – | – | 9·8 | 15·8 | 84·2 | 74·4 |
| | Means ± S.E. | 12·8 ± 0·65 | 37·3 ± 2·18 | 7·5 ± 0·57 | 211·0 ± 34·72 | 16·8 ± 3·68 | 18·5 ± 2·61 | 81·5 ± 2·60 | 64·8 ± 3·40 |
| | *Pregnant* Gestation (weeks) | | | | | | | | |
| 1 | 10–12 | 12·8 | 40·0 | 14·4 | 193·0 | 13·5 | 22·1 | 77·9 | 64·4 |
| 2 | 10–12 | 12·0 | 37·0 | 8·4 | 342·0 | 8·8 | 27·8 | 72·2 | 63·4 |
| 3 | 10–12 | 12·8 | 38·0 | – | – | 31·6 | 14·1 | 85·9 | 54·3 |
| 4 | 16–18 | 10·8 | 37·0 | 7·0 | 42·0 | 26·5 | 8·9 | 91·1 | 64·6 |
| 5 | 20–22 | 12·6 | 35·0 | – | – | 9·6 | 9·6 | 90·4 | 59·2 |
| 6 | 24–26 | 10·8 | 33·0 | 6·9 | 304·0 | 20·3 | 31·7 | 68·3 | 48·0 |
| 7 | 24–26 | 11·0 | 35·0 | 6·1 | 268·0 | 14·3 | 33·4 | 66·6 | 52·3 |
| 8 | 26–28 | 10·6 | 32·0 | 2·6 | 72·6 | 6·3 | 5·8 | 94·2 | 87·9 |
| 9 | 26–28 | 11·5 | 33·0 | – | – | 17·1 | 10·1 | 89·9 | 63·4 |
| | Means ± S.E. | 11·6 ± 0·41 | 35·6 ± 0·43 | 7·6 ± 1·58 | 203·6 ± 50·58 | 18·8 ± 3·12 | 18·1 ± 3·88 | 81·8 ± 3·62 | 61·9 ± 3·85 |

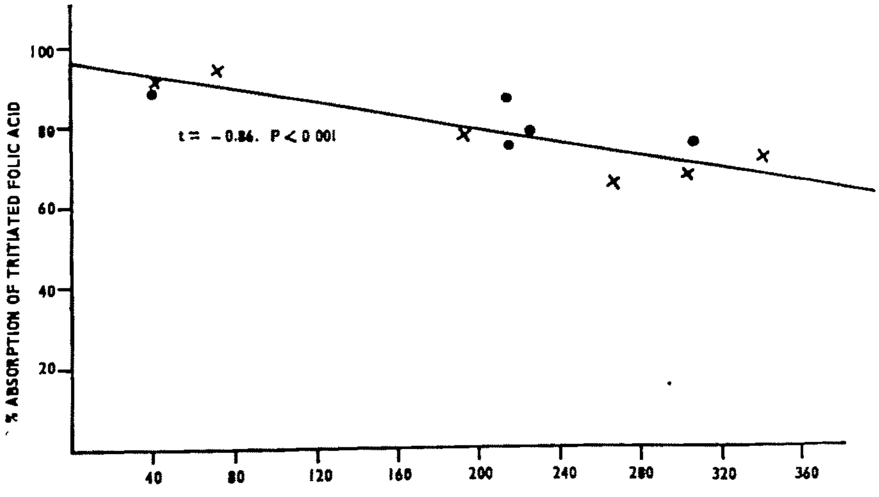

FIG. 1
Red cell folate levels and percentage absorption of tritiated folic acid.
× denotes pregnant subjects. ● denotes non-pregnant subjects.

absorption of polyglutamates was impaired in these subjects, that of monoglutamate was not altered. On the other hand, McClean *et al.* (1970) did not find any differences in the absorption of either mono- or polyglutamates in pregnancy, though the absorption of both was lower than the absorption in non-pregnant women. However, all the above studies had used crystalline compounds without taking into consideration the changes in blood volume consequent on pregnancy. Use of labelled compounds wherever feasible may provide more accurate information in this conflicting area.

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# RELATIONSHIP BETWEEN CONTINUOUS FETAL HEART RATE PATTERNS AND APGAR SCORE IN THE NEWBORN

BY

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## Summary

The relationship between the continuous fetal heart rate pattern in the first stage of labour and the one-minute Apgar score of the newborn was examined in 714 patients. The highest incidence of fetal depression (one-minute Apgar score less than 7) was observed with the late deceleration pattern and with variable deceleration associated with an abnormal baseline. Variable deceleration with a normal baseline and loss of beat-to-beat variation, both complicated and uncomplicated, were associated with infants with a low Apgar score to a greater degree than were the other patterns.

The continuous record of fetal heart rate (FHR) and uterine activity is gaining wider application in clinical obstetric practice. The patterns have been related to the mean Apgar score in high risk patients (Wood *et al.*, 1969) and in cases with fetal distress (Hobel, 1971). A scoring system based on the FHR pattern has been used to predict the Apgar score of the newborn (Hon, 1967; Schifrin and Dame, 1972). However, the incidence of depressed infants associated with each FHR pattern has only been reported in a series of 100 cases (Goodlin, 1971) and in 356 high risk patients (Beard *et al.*, 1971). Because the incidence of moderate depression (Apgar 4 to 6) or marked depression (Apgar 0 to 3) associated with each pattern is an important question in the management of an individual labour we have studied this relationship in 714 cases.

## Patients and Methods

The study was undertaken at a university affiliated County Medical Centre between 1st January 1972 and 31st December 1972. During this period there were 2413 total births and in 714 (29·6 per cent) satisfactory cardiotocographic tracings were obtained. Of the monitored patients, 475 had antepartum or intrapartum characteristics which placed them in a high-risk category. The remaining 239 patients were uncomplicated. A minimum of 30 minutes recording of FHR prior to full dilation of the cervix and uninterrupted recording after the record was started were used as criteria for inclusion in the study.

FHR and uterine activity were recorded with a commercially available fetal monitor (Cardiotocograph, Hewlett-Packard, Ltd.). The fetal heart rate was recorded using either a clip or spiral electrode attached to the presenting part in approximately 65 per cent of the cases. In the remainder, an abdominal ultrasound source was used to record FHR. In almost all cases uterine activity was recorded with an abdominal tocograph. A paper speed of 1 cm. per minute was used routinely and changed to 2 cm. per minute when an abnormal pattern was observed.

The tracings were classified according to the criteria of Beard *et al.* (1971) which was based on earlier work (Caldeyro-Barcia *et al.*, 1966;

Hon and Quilligan, 1968). These criteria are summarized in Table I. We have modified the classification of Beard *et al.* (1971) in a manner which avoids cross-classification but retains their criteria. As can be seen in Table I, complicated loss of beat-to-beat variation included only baseline complications (tachycardia or bradycardia). When loss of beat-to-beat variation was complicated by late deceleration we included these cases in the late deceleration group. When variable deceleration occurred with loss of beat-to-beat variation it was included only in the variable deceleration with abnormal baseline group. Thus each of the patients studied was placed in only one FHR category. The one departure from the criteria of Beard *et al.* (1971) is depicted in Figure 1. This pattern will be further discussed in the results.

## RESULTS

The distribution of FHR patterns in the 714 patients studied is given in Table II. Table II

TABLE I

*Criteria for classification of FHR patterns*

| FHR pattern | Criteria |
|---|---|
| Normal | Rate in the range 120 to 160 beats per minute; no change in rate throughout uterine contractions; beat-to-beat variation greater than five beats per minute. |
| Uncomplicated baseline tachycardia | FHR between contractions of 160 or more beats per minute; no change in rate throughout contraction; beat-to-beat variation greater than five beats per minute. |
| Uncomplicated baseline bradycardia | FHR between contractions of 120 or less beats per minute; no change in rate throughout contraction; beat-to-beat variation greater than five beats per minute. |
| Uncomplicated loss of beat-to-beat variation | Variation in FHR of five beats or less per minute; rate in the range 120 to 160 beats per minute, no change in rate throughout contractions. |
| Complicated loss of beat-to-beat variation | Variation in FHR of five beats or less per minute; rate of 160 or more or rate of 120 or less beats per minute. |
| Acceleration | FHR increases above the baseline rate during uterine contraction; rate in the range 120 to 160; beat-to-beat variation greater than five beats per minute. |
| Early deceleration | FHR decreases below baseline as the intrauterine pressure is rising and returns to baseline before the end of the contraction; amplitude of the deceleration does not exceed 40 beats per minute. |
| Late deceleration | FHR decelerations characterized by a lag time of at least 18 seconds; failure of FHR to return to baseline well after the end of the contraction; amplitude of the deceleration rarely exceeds 30 beats per minute. |
| Variable deceleration with normal baseline | FHR deceleration is variable in onset relative to the uterine contraction and variable in shape; FHR deceleration is abrupt with sharp return to baseline; baseline rate between 120 and 160 beats per minute; beat-to-beat variability greater than five beats per minute. |
| Variable deceleration with abnormal baseline | Baseline rate 160 or more, or 120 or less beats per minute; variation in FHR of five beats or less per minute. |

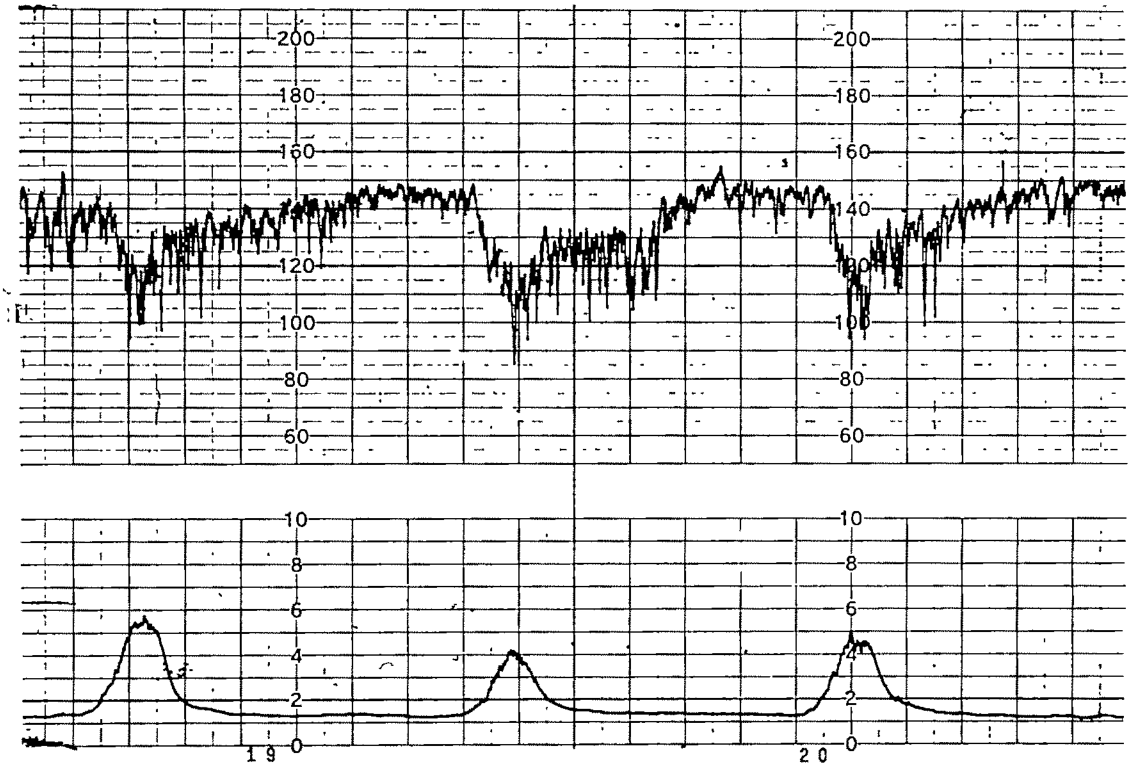

FIG. 1

Paper speed 2·0 cm. per minute. FHR deceleration occurs early in the uterine contraction phase but fails to return to the baseline at the end of the contraction.

TABLE II

*Relationship between FHR pattern and one-minute Apgar score and incidence of depression in the newborn*

| FHR pattern | Number | Per cent | Mean Apgar | Apgar 4–6 (moderate depression) |  | Apgar 0–3 (marked depression) |  |
|---|---|---|---|---|---|---|---|
|  |  |  |  | Number | Per cent | Number | Per cent |
| Normal | 322 | 45·0 | 8·2 | 16 | 5·0 | 3 | 0·9 |
| Uncomplicated baseline tachycardia | 24 | 3·4 | 7·9 | 1 | 4·2 | 1 | 4·2 |
| Uncomplicated baseline bradycardia | 38 | 5·3 | 8·1 | 3 | 7·9 | 0 | 0 |
| Uncomplicated loss of beat-to-beat variation | 40 | 5·6 | 7·2 | 7 | 17·5 | 2 | 5·0 |
| Complicated loss of beat-to-beat variation | 11 | 1·5 | 6·7 | 2 | 18·2 | 2 | 18·2 |
| Acceleration | 52 | 7·3 | 8·4 | 1 | 1·9 | 0 | 0 |
| Early deceleration | 122 | 17·1 | 8·0 | 6 | 4·9 | 1 | 0·8 |
| Late deceleration | 20 | 2·8 | 5·4 | 7 | 35·0 | 5 | 25·0 |
| Variable deceleration with normal baseline | 71 | 9·9 | 7·7 | 9 | 12·7 | 2 | 2·8 |
| Variable deceleration with abnormal baseline | 14 | 2·0 | 5·9 | 0 | 0 | 7 | 50·0 |

includes the mean one minute Apgar score associated with each pattern and the incidence of moderate depression (Apgar 4 to 6) and marked depression (Apgar 0 to 3) of the newborn related to the FHR pattern of the first stage of labour.

Four FHR patterns (uncomplicated baseline tachycardia; uncomplicated baseline bradycardia; acceleration; and early deceleration) were associated with a newborn condition which did not differ from that associated with a normal FHR pattern. When these four patterns were grouped with the normal pattern they accounted for 558 (78 per cent) of the patients monitored. The mean Apgar score at one minute in this group was 8·2 with a 4·8 per cent incidence of moderate depression and an 0·9 per cent incidence of marked depression.

Two FHR patterns were clearly associated with fetal depression. One-half of the 14 infants with variable deceleration and an abnormal baseline (this was usually tachycardia and/or loss of beat-to-beat variation) were markedly depressed and the mean one minute Apgar score of the whole group was 5·9. The late deceleration group (20 patients) had a mean Apgar score at one minute of 5·4 and 60 per cent had a score of 6 or less. The late deceleration group was not subdivided into those with and without an abnormal baseline. There were two patients in whom no lag time occurred but the FHR failed to return to baseline well after the uterine contraction. One of these is depicted in Figure 1. Both infants were markedly depressed (Apgar 0 and 3) and we have included this pattern in the late deceleration group.

The remaining three FHR patterns fell between the extremes of a vigorous and a depressed infant. Variable deceleration with a normal baseline, which made up close to 10 per cent of the study group, was associated with an Apgar score of 6 or less in 15·5 per cent of the group. Twenty-two per cent of infants delivered after an uncomplicated loss of beat-to-beat variation were depressed. When the flat FHR pattern was complicated by an abnormal baseline rate (this was almost always tachycardia) 36·4 per cent of the newborn infants had low Apgar scores.

There was one intrapartum fetal death among the 714 patients monitored. This involved a patient with undiagnosed twins. An abdominal FHR source was used and the initial segment showed late deceleration with loss of beat-to-beat variation. The FHR was then lost and the ultrasound head was replaced on the upper portion of the uterus with a normal FHR tracing resulting. At delivery the first twin was stillborn and the second twin had an Apgar score of 3 at one minute and 7 at five minutes.

## DISCUSSION

In this study we have chosen to relate the one minute Apgar score with the FHR pattern in the first stage of labour. While the five minute score is known to correlate best with subsequent neurological development, it does depend on the rapidity and skill of resuscitation. We have felt that the one minute Apgar score is a better index of the intrauterine state.

Since we have used the criteria of Beard *et al.* (1971) our results can best be contrasted to their report. The incidence of abnormal tracings in the present report is less than that of Beard *et al.* (1971) and also Wood *et al.* (1969). This may be explained by the patient population as these other two groups of workers reported on high-risk pregnancies while approximately one-third of our patients had no antepartum or intrapartum risk factors. We agree with Beard *et al.* (1971) in that the following five FHR patterns are associated with a good Apgar score in the newborn: normal; uncomplicated baseline tachycardia; uncomplicated baseline bradycardia; acceleration; and early deceleration. In the King's College Hospital group (Beard *et al.*, 1971) the incidence of low Apgar scores ranged from 16 to 21 per cent in these patterns. This was attributed, in part, to operative delivery. In our patients depression was seen in only 1·9 to 8·4 per cent and was less than 6 per cent in the entire group. In one year we monitored close to 30 per cent of deliveries and nearly 80 per cent of tracings showed normal patterns and no further intervention in labour was necessary.

The clear relationship between late deceleration and fetal depression confirms the work of others (Beard *et al.*, 1971; Wood *et al.*, 1969; Hobel, 1971). This is also true of variable deceleration when it is associated with an abnormal baseline (Beard *et al.*, 1971; Wood *et al.*, 1969). Goodlin (1971) has reported that loss of beat-to-beat variation associated with a normal rate or with bradycardia is associated with the normal 10 per cent incidence of Apgar scores below 7. However, in the study by Beard *et al.* (1971) the flat uncomplicated trace was associated with depression in 25 per cent and in the present study in 22·5 per cent of newborn infants. When the loss of beat-to-beat variation was complicated especially by tachycardia then there was agreement between the three studies. Goodlin (1971) reported two patients with tachycardia and both infants were depressed. Beard *et al.* (1971) reported depression in 15 of 19 infants with this pattern as contrasted to 4 of 11 in the present study. However, as noted above, the King's College Hospital group included decelerations as complications of loss of beat-to-beat variation. Thus, 5 patients had late deceleration and two had variable deceleration with tachycardia. In the present study these patients would have been classified in their respective deceleration categories.

This study was conducted during the first year of systematic fetal monitoring on the obstetrical service. During this period the house officers became more familiar with the patterns and on the basis of the results a plan of clinical management evolved. When one of the five patterns associated with a satisfactory one minute Apgar score in the newborn is present no further investigations are made. When late deceleration or variable deceleration with an abnormal baseline are present (4·8 per cent of cases) fetal scalp blood is obtained for pH determination. Timing of delivery is then dependent on the presence or absence of fetal acidosis. The loss of beat-to-beat variation patterns and variable deceleration with a normal baseline are also indications for fetal scalp sampling, but the urgency is less than with the two patterns which are more strongly associated with fetal depression. Because of its descriptive nature we have found that the classification of Beard *et al.* (1971) to be excellent in education of resident physicians and in management of labour in clinical practice.

## ACKNOWLEDGEMENTS

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# BIOLOGICAL ASPECTS OF TWIN PREGNANCY IN DUNDEE

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## Summary

A review is presented of 712 twin pregnancies which occurred in Dundee between 1956 and 1971. It shows a falling age of twin reproduction, a changing ratio of mono- to dizygosity and the poor growth rates of both types. Birth weight variations suggest that "crowding" in the uterus may affect the individual growth of each twin, but the combined weight of twins continues to increase to term and shows no obvious sign of restriction.

ALTHOUGH one out of every eighty pregnancies is a twin pregnancy many aspects of this common phenomenon remain unclear. Dizygotic twinning, the result of multiple ovulation, is the most common, its frequency varying with such factors as race, nationality and maternal age.

Benirschke and Kim (1973) reviewed all the known factors. These included differing hormonal balances as a cause of dizygotic twinning with racial variation in gonadotrophin production by the pituitary in the stimulation of the ovaries. Reinforcing this is the fact that multiple ovulation has been induced in women who have been treated with gonadotrophin or gonadotrophin-releasing agents. Women who conceive within three months of marriage have been observed to have a greater frequency of twins and it has been reported that dizygotic twinning increases with maternal age, reaching its peak at between 35 and 39 years and then dropping sharply. Other studies have suggested that twinning may be influenced by season and light exposure, and civil state with a "peak" in unmarried mothers between the ages of 25 and 29.

As yet there are no valid hypotheses as to the cause of monozygotic twinning. Unlike dizygotic twinning the incidence is not apparently influenced by race, maternal age, nutrition or any other factor, and its frequency appears to be relatively constant throughout the world.

The review of Ounsted and Ounsted (1973) indicated that two fetuses might together present needs which the uterine blood supply could not meet and that therefore multiple pregnancy must be treated as quite a separate issue in terms of fetal growth rate.

### MATERIAL

In the Dundee general hospitals between 1956 and 1971 there were 712 twin pregnancies. From the 712 twin pregnancies 40 had to be excluded because they were like-sex twins where the state of the membranes was not recorded and zygosity studies had not been undertaken. We have used the policy of Benirschke (1961) for recording zygosity of multiple births. All multiple pregnancy placentae were accurately labelled during delivery by markers on each umbilical cord. No placenta was discarded until all results were known, storage being in fixative or in a deep freeze. The gross appearances of placentae from like-sexed twins whether fused, partially fused or separate, were noted and used to decide whether the babies were dizygotic or mono-

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zygotic. Membrane examination recorded whether two, three or four membranes are present. By this method dizygotic twins were excluded and the final group of like-sexed twins with monochorial or dichorial placentae were subjected to blood grouping studies so that a final accurate record of monozygosity could be reached.

This policy meant that, since 1961, only in 12 cases (out of over 400 in Dundee) was the zygosity not accurately recorded.

A further 247 pairs were excluded on the grounds that both twins were not born alive, or that the period of gestation was not accurately known, leaving 140 monozygotic and 285 dizygotic twin pregnancies.

## FINDINGS

### *Incidence*

In the 16 years 1956 to 1971 there were in Dundee 53 266 registered deliveries. Of the 712 sets of twins all but 91 were born to Dundee resident mothers. During the same period there were 6 triplet pregnancies. Thus we derive a ratio of twin to single pregnancy (1 : 86) and triplet to twin (1 : 103) for Dundee resident mothers.

### *Maternal age*

Table I shows a maximum incidence of monozygotic twins in mothers less than 19 years of age and a peak incidence of dizygotic twins in mothers aged 30 to 34.

TABLE I

*Twin pregnancy related to maternal age (1956 to 1971)*

| Mother's age | Monozygotic | Dizygotic | Total |
|---|---|---|---|
| 19 and less | 20<br>(0·40) | 21<br>(0·40) | 41 |
| 20 to 24 | 53<br>(0·28) | 80<br>(0·42) | 133 |
| 25 to 29 | 34<br>(0·21) | 80<br>(0·57) | 114 |
| 30 to 34 | 20<br>(0·22) | 73<br>(0·83) | 93 |
| 35+ | 13<br>(0·23) | 31<br>(0·61) | 44 |
| Total | 140<br>(0·26) | 285<br>(0·53) | 425 |

Figures in parentheses represent percentage incidences of total births in that age group.

TABLE II

*Twin pregnancy related to parity (1956 to 1971)*

| | Previous pregnancies | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4+ |
| Monozygotic | 46<br>(0·6) | 40<br>(0·5) | 19<br>(0·5) | 14<br>(0·3) | 21<br>(0·4) |
| Dizygotic | 57<br>(0·6) | 54<br>(0·7) | 57<br>(0·8) | 68<br>(0·9) | 49<br>(0·8) |
| Total | 103<br>(1·2) | 94<br>(1·2) | 76<br>(1·3) | 82<br>(1·2) | 70<br>(1·2) |

Figures in parentheses represent percentage incidence of total births by parity.

### *Parity*

Table II shows that the incidence of monozygotic and dizygotic twins is approximately the same for each parity group.

### *Growth rates*

For the fully characterized 425 twin pairs birthweight was plotted against the completed weeks of gestation for both monozygotic and dizygotic twins. The comparison is made between these figures and those for single pregnancy percentiles for the Dundee population (Walker, 1967); the poor growth record of both types of twins is seen (Fig. 1). The mean weight of each type of twin pregnancy is similar to that of a single fetus up to about 29 weeks (i.e. comparable with the 50th percentile of singleton pregnancies) but thereafter the growth rate falls progressively behind that of singleton fetuses until after the 37th week the mean is below the 10th percentile for the single fetus.

When constructing our growth curve, cases of pre-eclamptic toxaemia and antepartum haemorrhage in the mother were included because they were so few in number that they could not be extracted to give a separate curve.

### *Birthweight variations*

Figure 2 shows birthweight plotted for monozygotic and dizygotic twins on the basis of *weight difference* between twin 1 and twin 2.

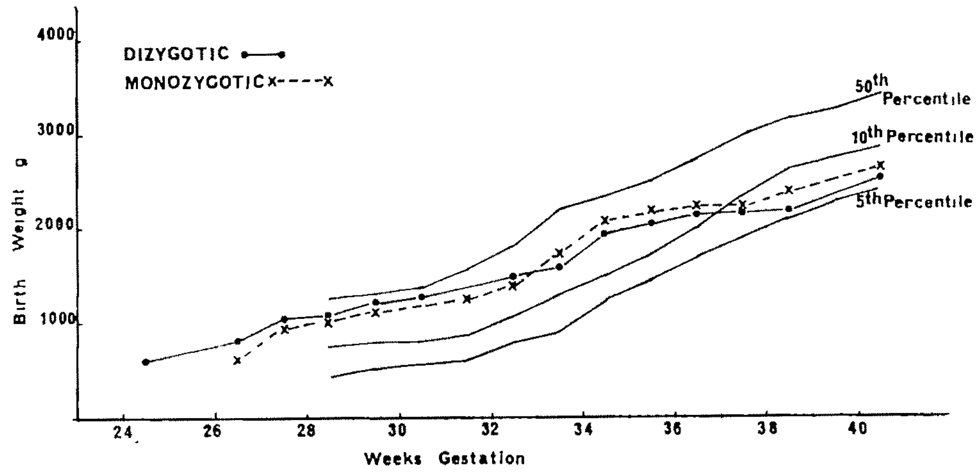

FIG. 1
Growth of twins compared with singleton pregnancies 50th, 10th and 5th percentiles.

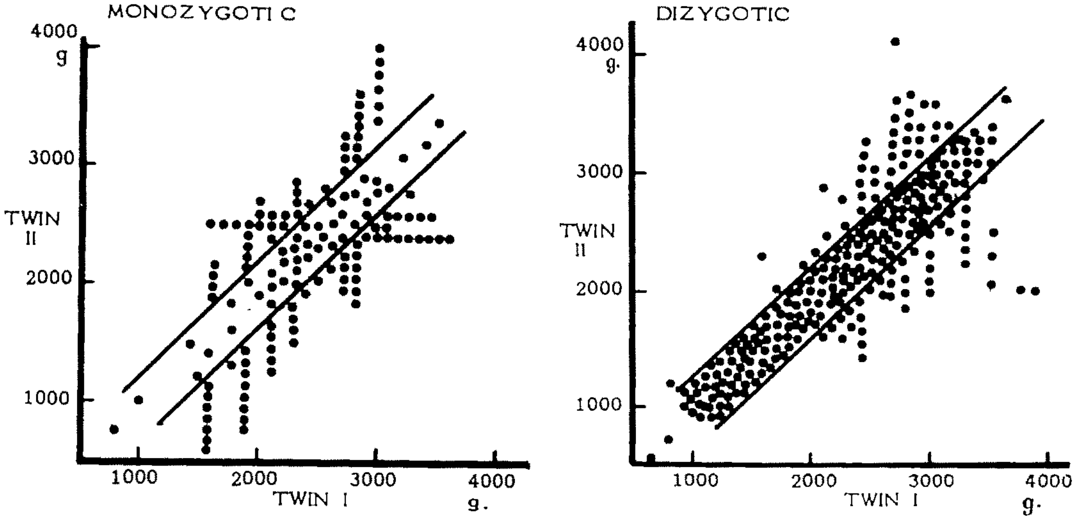

FIG. 2
Variations in twin pregnancy birthweights. Area between solid lines represents weight difference of 440 g.

Monozygotic twins showed a much wider scatter and the ratio of weight differences (greater than 440 g. less than 440 g. was approximately 2 : 1. This being the ratio of the 43 cases within the limits shown by the two solid lines, and the 97 outside the lines. Dizygotic twins were much less scattered and the ratio of weight differences (greater than 440 g. less than 440 g.) was reversed at 1 : 2. In Figure 2 this is shown by 203 cases between the lines and only 82 outside.

The *combined weights* and the *biggest weight difference* for pairs of twins, in our series, was similar for both monozygotic and dizygotic pairs. For monozygotic the greatest combined weight was 3700 plus 4055 (or 7755) g., and for dizygotic 3980 plus 3580 (or 7560) g. The greatest difference between individuals for monozygotic twins was 3780 minus 2160 (or 1620) g., and dizygotic 4130 minus 2370 (or 1760) g.

In Figure 3 we have plotted the combined weights of 425 twin pairs against completed

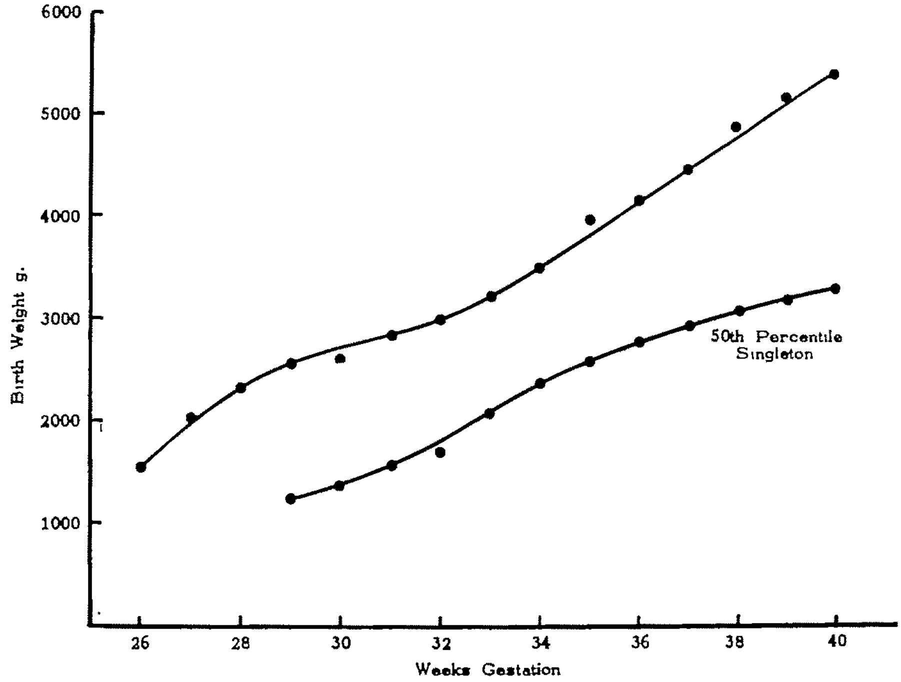

FIG. 3
Combined mean weight of twin pair against completed weeks of pregnancy.

weeks of pregnancy. For comparison the 50th percentile for singleton pregnancies in Dundee are added (Walker, 1967). As parity and zygosity have little effect upon the incidence of twinning (see above) and hence upon the weight of the conceptions, no breakdown into parity or zygosity was made.

*Combining* the weights of the twin pair masks the poor growth rates recorded in Figure 1 for the *mean* weight of individual twins.

### *Maternal weight for height variations*

In an attempt to explore possible factors involving the "uterine crowding" theory of McKeown and Record (1952) we compared the incidence of monozygotic and dizygotic twins in mothers who were classified as of low, standard or high body weight, using the weight-for-height standards of Kemsley *et al.* (1962). To achieve a reasonably representative non-pregnant weight for the mother, we only used patients who attended the booking clinic when less than 16 weeks pregnant. Of the 425 cases only 210 fell into this category, of which 100 were primigravidae. This analysis showed that monozygotic and dizygotic twins occur in approximately the same ratio regardless of parity in each of the three standard maternal weight-for-height groups, but that the underweight mother produces lower weight twins and the overweight mother produces higher weight twins, independent of the zygosity. "Uterine crowding" may therefore purely relate to the fact that the smaller woman has a smaller uterus and smaller twins.

## DISCUSSION

A discussion of the biological aspects of twin pregnancy gives the opportunity of comparing two different clinical phenomena with a common result. The incidence of monozygotic and dizygotic twins in Dundee is similar to previously published reports, but the incidence hides subtle changes.

Previous reports of twin pregnancy have shown that maternal age influences the incidence with a peak in the age group 35 to 39 years (Waterhouse, 1950; Benirschke and Kim, 1973). However, Guttmacher (1953) suggested that the age of twin reproduction was falling. Our figures lend support to this view, especially as regards dizygotic twins, and we believe that we are not alone in this view; it is reflected in the latest Scottish National figures (compare Registrar General Reports, 1965 to 1969 with 1970 to 1972).

When compared with the world figures (Hytten and Leitch, 1971) our figures do not show such a steep rise of dizygotic twinning with age, and our monozygotic figures show a maximum incidence in the youngest age group and a fairly constant level thereafter. Das (1930) claimed that twinning more commonly occurred in young primigravidae but Waterhouse (1950) concluded "the incidence of monozygotic twins is virtually independent of maternal age . . . the incidence of dizygotic twins rises sharply . . . and falls".

Hytten and Leitch (1971) reviewed the conflicting reports of the influence of parity upon the incidence of twinning. The conclusion of Waterhouse (1950) would appear to be the most accurate: "the effect of birth rank is merely to cause a virtual displacement of the curve, which summarizes the effects for all birth ranks."

Our figures would seem to suggest that the two factors of an increasingly younger reproductive age group and the limitation of family size are acting together to produce a fairly constant annual incidence of twinning. The long-term effect of this may well produce a situation in which the incidence of dizygotic twins falls to that of monozygotic twins.

In his study on half siblings, twins, full siblings and consanguineous matings, Morton (1955) concluded that the resemblance in birthweight of siblings was largely attributable to the maternal constitution or environment and not to genetic similarity.

The poor performance by both monozygotic and dizygotic twins as regards growth rates was noted by Gruenwald (1970). Our work substantiates the point made by McKeown and Record (1952) who suggested that "the mean fetal weight is independent of litter size until about the 27th week of gestation, that the rate of growth of individual multiple fetuses is slower than that of the single fetus, from various stages of gestation and that retardation of fetal growth may be attributable to crowding in the uterus and may be imposed through the placenta". They noted that at the time from which the respective rates of fetal growth are retarded the mean litter weight of the twin, triplet or quadruplet was approximately the same.

Our birthweight variations tend to support the "uterine crowding" theory of McKeown and Record (1952). A weight difference of more than 440 g. between twins is more characteristic of monozygotic than dizygotic twins. It could be that dizygotic twins achieve a better degree of sharing in the placenta than monozygotic twins. In the study by Morton (1955) the similarity of the like-sexed and unlike-sexed twin correlations was taken as evidence that the resemblance between twins was due to common uterine environment (and not genetic relationship) and was twice as great for monozygotic as for dizygotic twins.

While the "uterine crowding" hypothesis of McKeown and Record (1952) is supported by the poor growth rate of individual twins, it is less easy to reconcile with the evidence of the combined weight of the two twins, when a total weight of over 5000 g. is achieved at term with no evidence, comparable to the well known pattern in singletons, of a final slowing of growth. Our maternal weight-for height-variations suggest that the smaller woman, with presumably a smaller uterus, has smaller twins, but there is no evidence that uterine capacity is responsible for restriction of growth.

## ACKNOWLEDGEMENTS

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# BLOOD COAGULATION PROFILE IN INDIAN PATIENTS WITH PRE-ECLAMPSIA AND ECLAMPSIA

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## Summary

Twelve Indian patients with pre-eclampsia, 15 with eclampsia and 15 with normal pregnancy in the third trimester were investigated. A systemic bleeding diathesis was encountered in two patients with eclampsia and in none with pre-eclampsia; two patients with pre-eclampsia, however, had excessive uterine haemorrhage. Coagulation studies showed statistically significant prolongation of thrombin time, elevation of serum fibrinogen degradation products (FDP) and hypofibrinogenaemia in patients with pre-eclampsia as well as eclampsia. In patients with eclampsia, significant thrombocytopenia also occurred. Euglobulin lysis time showed no significant change in patients with pre-eclampsia and eclampsia. There was no significant difference in the coagulation profile between patients with eclampsia and pre-eclampsia, except for more hypofibrinogenaemia in the former. The laboratory findings suggest the occurrence of intravascular coagulation in patients with pre-eclampsia and eclampsia.

ALTHOUGH a major haemorrhagic diathesis seldom occurs in patients with pre-eclampsia and eclampsia, these patients often exhibit a tendency formin or haemorrhages (Kennan *et al.*, 1957). At autopsy, patients dying of pre-eclampsia and eclampsia often show haemorrhagic areas in the liver, lungs, adrenals, serous membranes and gastrointestinal tract (McKay, 1965). Data on coagulation factors and the fibrinolytic state in pre-eclampsia and eclampsia have been reported (Schneider, 1951; Wardle and Menon, 1969; Bonnar *et al.*, 1971; Howie *et al.*, 1971). Recently evidence for the occurrence of disseminated intravascular coagulation has been put forward (McKay, 1965; Hjort and Rapaport, 1965; Vassalli and McCluskey, 1965) and has been questioned by Sophian (1971). It was considered worth reporting our coagulation data in 27 patients with pre-eclampsia or eclampsia.

## METHODS

The present study was performed on 15 patients with a normal third trimester pregnancy, 12 patients with pre-eclampsia and 15 with eclampsia. A diagnosis of pre-eclampsia implied the presence of hypertension, albuminuria (detected by boiling urine) and oedema. Eclampsia was diagnosed when fits were associated with these signs. All patients with pre-eclampsia or eclampsia had a blood pressure of at least 150/100 mm. Hg.

Routine haematological tests were done as described by Dacie and Lewis (1968). Coagulation tests were mostly done by the methods described by Denson (1966). The other measurements made were plasma fibrinogen (Quick, 1966), euglobulin lysis time (Buckell, 1958), thrombin time (Owen *et al.*, 1969) and the serum fibrinogen degradation products (FDP) (Merskey *et al.*, 1969). The differences between groups were statistically noted by using the Student's "t" test.

## RESULTS

Twelve patients with pre-eclampsia and 15 with eclampsia were studied. The age, parity and period of gestation of these patients are given in Table I. One patient with pre-eclampsia had antepartum haemorrhage while another had antepartum and postpartum haemorrhage; there were no other manifestations of systemic bleeding in patients with pre-eclampsia. However, two patients with eclampsia had multiple cutaneous ecchymoses, bleeding from the mouth and gums, haematemesis, haematuria and postpartum haemorrhage. One of them probably died from pontine haemorrhage. Haematuria was observed in three additional patients. The results obtained in all patients are summarized in Table II.

TABLE I

*Age, parity and period of gestation of patients with pre-eclampsia and eclampsia*

| | Patients with | | | |
|---|---|---|---|---|
| | Pre-eclampsia | | Eclampsia | |
| | No. | Per cent | No. | Per cent |
| *Age:* | | | | |
| 20–29 | 1 | 8·3 | 6 | 40·0 |
| 30–39 | 10 | 83·3 | 8 | 53·3 |
| 40–49 | 1 | 8·3 | 1 | 6·7 |
| *Parity:* | | | | |
| Primiparae | 8 | 66·7 | 7 | 46·7 |
| Secundiparae | 2 | 16·7 | 4 | 26·7 |
| Multiparae | 2 | 16·7 | 4 | 26·7 |
| *Period of gestation:* | | | | |
| 28th to 32nd week | 4 | 33·3 | 4 | 26·7 |
| 33rd to 36th week | 4 | 33·3 | 6 | 40·0 |
| 37th to 40th week | 4 | 33·3 | 5 | 33·3 |

### *Coagulation profile in patients with pre-eclampsia*

Bleeding time, whole blood clotting time and prothrombin time ratio were within normal limits in all patients. Thrombin time ratio was more than 1·2 in 6 patients (50 per cent); in all these patients the thrombin time ratio was only partially corrected after addition of equal amount of normal plasma. Euglobulin lysis time was less than 170 minutes in five patients. The platelet count was less than 150 000 per cu. mm. in three patients. The serum FDPs were more than 10 μg./ml. in 2 out of 12 patients; one of them had a value of 40 μg./ml.

### *Coagulation profile in patients with eclampsia*

Bleeding time and whole blood clotting time were in the normal range in all patients. The prothrombin time ratio was more than 1·2 in

TA

*Coagul*

| | | | Euglobulin lysis time (minutes) | Platelet count (per cu. mm.) | Prothrombi consumption in (per cent) |
|---|---|---|---|---|---|
| Patients with pre-eclampsia | (n = 12) | Mean<br>±SD | 160·4<br>±40·9 | 195,000<br>±61,890 | 8·91<br>±1·69 |
| Patients with eclampsia | (n = 15) | Mean<br>±SD | 166·7<br>±43·6 | 181,800* ↓<br>±60,410 | 9·96<br>±2·02 |
| Normal patients in third trimester | (n = 15) | Mean<br>±SD | 212·7<br>±33·3 | 236,400<br>±42,890 | 9·42<br>±2·81 |

* ↑ Significantly higher than mean normal value (p<0 05).
** ↑ Very significantly higher than normal value (p<0·01).
* ↓ Significantly lower than normal value (p<0·05)
** ↓ Very significantly lower than normal value (p<0·01).

one of the patients. The thrombin time ratio was more than 1·2 in 10 out of the 15 patients. In 8 out of these 10 patients thrombin time ratio was partially corrected after the addition of an equal volume of normal plasma, but no significant correction was noted in two patients. The euglobulin lysis was markedly enhanced in one patient in whom it was only 90 minutes. The kaolin cephalin clotting time (KCCT) ratio was more than 1·2 in seven patients. The platelet count was less than 150 000 per cu. mm. in three patients. The serum FDPs were raised in 10 patients; in four of them they were 40 μg./ml. or more.

The mean values together with standard deviation of the different coagulation tests in normal pregnancy (15 women in the third trimester) in pre-eclampsia and in eclampsia are shown in Table II. On making statistical comparisons between mean levels in patients with pre-eclampsia and normal patients in the third trimester, no significant difference was found in the bleeding time, the whole blood clotting time, the prothrombin time, the euglobulin lysis time, the kaolin cephalin clotting time, the prothrombin consumption index and the platelet count. However, significant increases were found in thrombin time ratio (p<0·05) and serum FDPs (p<0·01) while the plasma fibrinogen was significantly reduced (p<0·05).

On making statistical comparisons between mean values in patients with eclampsia and those with normal third trimester pregnancy, no significant differences were found in the prothrombin time, the kaolin cephalin clotting time, the euglobulin lysis time and the prothrombin consumption index. There was a significant increase in the bleeding time (p<0·01), the whole blood clotting time (p<0·01), the thrombin time ratio (p<0·01) and the serum FDP levels (p<0·01), while the platelet count was significantly reduced (p<0·05) as was the plasma fibrinogen (p<0·01).

The only significant difference between patients with pre-eclampsia and eclampsia was a reduction (p<0·05) in plasma fibrinogen in patients with eclampsia.

## DISCUSSION

The chief coagulation disturbances in pre-eclampsia and eclampsia were a significant prolongation of thrombin time, a reduction of plasma fibrinogen and a rise in serum FDP levels. Patients with eclampsia also showed a significant thrombocytopenia. The Birmingham Eclampsia Study Group (1971) found a significant reduction of plasma fibrinogen, plasminogen and blood platelets in 10 out of 19 patients with pre-eclampsia and in 6 out of 8 patients with eclampsia. They noted no significant difference in coagulation and fibrinolytic activity between pre-eclamptic and eclamptic patients. Dieckmann (1936) found significantly reduced levels of plasma fibrinogen in fatal cases of eclampsia.

Thrombocytopenia was recorded in patients with pre-eclampsia or eclampsia by other

*ameters*

| Plasma fibrinogen (mg. per ml.) | Serum FDPs (μg. per ml.) | Bleeding time (minutes) | Whole blood clotting time (minutes) | KCCT ratio | Prothrombin time ratio | Thrombin time ratio |
|---|---|---|---|---|---|---|
| 367·1* ↓<br>±37·2 | 11·7** ↑<br>±9·8 | 2·8<br>±0·8 | 3·6<br>±0·7 | 1·10<br>±0·68 | 1·03<br>±0·03 | 1·38* ↑<br>±0·41 |
| 322·5** ↓<br>±61·0 | 19·50** ↑<br>±21·55 | 3·1** ↑<br>±0·7 | 4·3** ↑<br>±0·8 | 1·15<br>±0·33 | 1·11<br>±0·21 | 1·34** ↑<br>±0·26 |
| 420 0<br>±79·3 | 3·00<br>±3·30 | 2·3<br>±0·5 | 3·2<br>±0·5 | 1·02<br>±0·11 | 1·03<br>±0·07 | 1·06<br>±0·07 |

workers (Kennan *et al.*, 1957; Morris *et al.*, 1964; Nielson, 1969; Howie *et al.*, 1971) and was attributed to probable consumption of platelets during disseminated intra-vascular coagulation (DIC). Increased platelet adhesiveness (McKay *et al.*, 1964) and persistently impaired platelet disaggregation (Howie *et al.*, 1971) are considered to be factors predisposing to DIC.

Though the euglobulin lysis time was shortened in about half of our patients, the change was not statistically significant. Other workers found the same in patients with pre-eclampsia (Nielsen, 1969; Wardle and Menon, 1969). However, Howie *et al.* (1971) found that the physiological depression of fibrinolytic activity occurring in pregnancy was slow to return to normal after delivery in several patients with severe pre-eclampsia. Bonnar *et al.* (1971) found significant depression of euglobulin lysis together with decreased urokinase sensitivity in severe pre-eclampsia.

High serum FDP levels in our patients with pre-eclampsia and eclampsia are in agreement with the findings of others (Henderson *et al.*, 1970; Birmingham Eclampsia Study Group, 1971; Bonnar *et al.*, 1971; Howie *et al.*, 1971). Significantly raised serum FDPs, thrombocytopenia and decreased plasma fibrinogen levels in association with insignificant increase in fibrinolytic activity in the present study indicate the occurrence of intravascular coagulation in patients with pre-eclampsia or eclampsia. The same is suggested by reports of raised levels of plasma cryofibrinogen (McKay and Corey, 1964; Wardle and Menon, 1969) and reduction in the mean plasminogen level in severe pre-eclampsia (Bonnar *et al.*, 1971; Howie *et al.*, 1971).

The other studies supporting the hypothesis of intravascular coagulation, have been summarized in a review by Coopland (1969). Immunofluorescent studies of renal biopsy specimens (Vassalli *et al.*, 1963; Morris *et al.*, 1964) have shown striking accumulation of fibrinogen derivatives within the swollen intracapillary cells. Gamma-globulins and complement are usually not demonstrable thus providing strong evidence against an immunologic basis for the lesion (Mellors *et al.*, 1957). McKay *et al.* (1953) found microthrombi in organs of patients dying of eclampsia and argued that these microthrombi did not represent an agonal phenomenon since there were simultaneous alterations in the clotting mechanism. Further, Ingerslev and Teilum (1946) reported fibrin thrombi in periportal regions of the liver of patients who survived eclamptic episodes. Lung scans in three out of five patients with eclampsia and three patients with pre-eclampsia showed impairment of basal flow and patchy areas of impaired perfusion (Birmingham Eclampsia Study Group, 1971).

Whether the changes found in these investigations are related to the pathogenesis of toxaemia of pregnancy or are the effect of the disease process cannot at present be stated (Bonnar *et al.*, 1971). Nevertheless, the current evidence about intravascular coagulation raises the question of the possible therapeutic value of anti-thrombotic agents in pre-eclampsia and eclampsia. On a theoretical basis, such treatment might be expected to improve the blood flow to the placenta and thereby the fetal growth. Reid *et al.* (1971) gave heparin to two patients with pre-eclampsia with a hypercoagulable state, and found improvements in the parameters studied, including disappearance of a fibrin monomer. The value of such anticoagulant therapy requires careful evaluation and may well be outweighed by the risks.

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# TOTAL URINARY OESTROGEN EXCRETION DURING NORMAL MENSTRUAL CYCLE AND NORMAL PREGNANCY IN NIGERIANS

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## Summary

Total oestrogens were measured during normal menstrual cycles and during pregnancy in healthy Nigerian women. During the menstrual cycle, maximum excretion was consistently observed in the luteal phase in contrast to findings in European and American populations. During pregnancy, oestrogen excretion was similar to that reported amongst Europeans only in early pregnancy; oestrogen excretion did not show the usual rapid rise in late pregnancy.

SOME geographic variations have been reported in the normal patterns of hormone secretion and excretion and attempts have been made to correlate these with the prevalence of liver dysfunction (Boulle, 1968) or a predisposition to carcinoma of certain target organs in the geographic areas concerned (Wang *et al.*, 1966; MacMahon *et al.*, 1971).

There is as yet limited information on the pattern of hormone secretion or excretion in African populations and even less about the sex hormones. Such information is, however, vital in view of the global interest now being directed towards the understanding and control of reproductive function. The present communication gives information on the pattern of total oestrogen excretion in pregnancy and during normal menstrual cycles of adolescent and mature nulliparous Nigerians.

## METHODS

The first group studied consisted of 105 schoolgirls between the ages of 12 and 19 who were boarders in two schools in the city of Ibadan. Each subject provided two samples of 24-hour urine, the second being provided fourteen days after the first urine collection. All the subjects had established menstrual periods.

The second group consisted of eight girls between the ages of 18 and 22 who were employees of the University College Hospital. Each subject provided 24-hour urine samples weekly over a period of three months. Thus a total of 23 normal cycles were studied. All the subjects had established periods and the average length of the cycle was 27·5 days (range 26 to 29). None of the subjects in either group was known to have a history of menstrual disorder or to be on oral contraceptives.

The third group consisted of 103 healthy women who were attending the ante-natal clinic of Inalende Maternity Centre, Ibadan. Of these 76 had uncomplicated deliveries of a single fetus at the maternity centre. The results of only these are used in the present report. Each subject provided one sample of 24-hour urine.

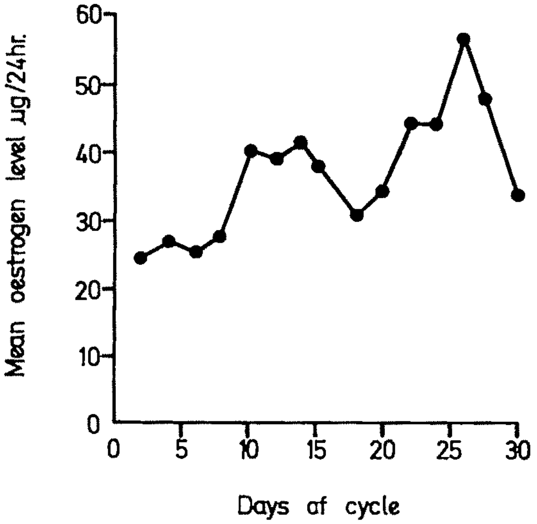

FIG. 1

Total urinary oestrogen levels during the menstrual cycle in 105 schoolgirls aged between 12 and 19 years. Day 1 is taken as the first day of menstruation.

TABLE I

*Total urinary oestrogen levels during the menstrual cycle in 105 schoolgirls aged between 12 and 19 years. Day 1 is taken as the first day of menstruation*

| No. of samples | Day of cycle | Oestrogen level (µg./24 hours) Mean±SD |
|---|---|---|
| 14 | 1, 2 | 24·8± 7·0 |
| 16 | 3, 4 | 26·9±11·6 |
| 10 | 5, 6 | 25·5±10 0 |
| 10 | 7, 8 | 27·7± 8·1 |
| 12 | 9, 10 | 40·5±12·9 |
| 18 | 11, 12 | 39·3±16 4 |
| 18 | 13, 14 | 41·8±20·1 |
| 14 | 15, 16 | 36·2±10·1 |
| 18 | 17, 18 | 31·2±11·9 |
| 14 | 19, 20 | 34·4±12 6 |
| 12 | 21, 22 | 44·7±30 9 |
| 10 | 23, 24 | 44·2±16·2 |
| 20 | 25, 26 | 57 3±30·5 |
| 10 | 27, 28 | 44·6±13·0 |
| 14 | 29, 30 | 33 1±11·1 |

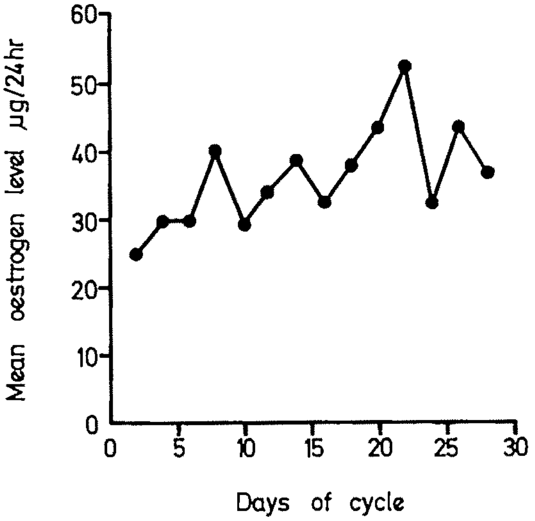

FIG. 2

Total urinary oestrogen levels during the 23 normal menstrual cycles in eight girls aged between 18 and 22 years. Day 1 is taken as the first day of menstruation.

Urinary oestrogens were extracted using the Paton-Brown semi-automatic extractor and the total urinary oestrogen levels in pregnancy were measured by colorimetry using the Kober reaction and in non-pregnancy by fluorimetry using the Kober-Ittrich procedures as described by Brown *et al.* (1968).

## RESULTS

Figure 1 shows the composite findings for the subjects in Group 1. The results (Table I) show a rise in oestrogen excretion in the follicular phase to a peak occurring between days 10 and 14 which was followed by a mid-cycle drop occurring about days 17 and 18, with a second rise in the mid-luteal phase and a final drop before the next menstruation. Figure 2 shows the results for the subjects in Group 2. The results are essentially similar to those of Group 1 (Tables I and II). The mean values (±standard deviation) for the pre-ovulatory maxima were 40·5±12·9 and 40·1±14·9 µg./24 hours and the mid-luteal maxima were 57·3±30·5 and 52·3±24·6 µg./24 hours for Groups 1 and 2 respectively.

In the pregnant subjects oestrogen excretion increased sharply from a mean value of 3·2±1·1 mg./24 hours at 18 weeks after the last menstrual period to a value of 15·9±8·1 mg./24 hours at the 26th week. A more gradual increase

TABLE II

*Total urinary oestrogen levels during 23 normal menstrual cycles in eight girls aged between 18 and 22 years. Day 1 is taken as the first day of menstruation*

| No. of samples | Day of cycle | Oestrogen level (μg./24 hours) Mean±SD |
|---|---|---|
| 7 | 1, 2 | 25·2±10·0 |
| 4 | 3, 4 | 29·8± 7·9 |
| 4 | 5, 6 | 29 8±19·2 |
| 13 | 7, 8 | 40·1±14·9 |
| 5 | 9, 10 | 29·5± 8·6 |
| 3 | 11, 12 | 34·6± 1·8 |
| 11 | 13, 14 | 39 8±13·7 |
| 7 | 15, 16 | 32·4± 8·1 |
| 2 | 17, 18 | 35·5 |
| 11 | 19, 20 | 43·4±14·5 |
| 9 | 21, 22 | 52·3±24·6 |
| 3 | 23. 24 | 32·7± 6·1 |
| 5 | 25, 26 | 44·2± 4·8 |
| 7 | 27, 28 | 35·0± 8 9 |

occurred from the 28th week to a maximum value of 27·3±10·8 mg./24 hours at term (Table III and Fig. 3).

TABLE III

*Total urinary oestrogen levels during normal pregnancy in 76 subjects*

| No. of samples | Weeks after last menstrual period | Oestrogen level (mg./24 hours) Mean±SD |
|---|---|---|
| 5 | 18 | 3·2± 1·1 |
| 6 | 20 | 5·5± 1·6 |
| 5 | 22 | 11·7± 5·7 |
| 12 | 24 | 15·9± 8·1 |
| 7 | 26 | 14·7± 9·3 |
| 13 | 28 | 18·1± 8·5 |
| 4 | 30 | 19·6± 9·4 |
| 17 | 32 | 20·8± 3·4 |
| 1 | 34 | 19·6 |
| 3 | 36 | 21·8± 4·7 |
| 3 | 38 | 27·3±10·8 |

## DISCUSSION

The pattern of urinary oestrogen excretion as observed in this study is essentially the same as those described in other communities, for example in Swedish populations (Johansson *et al.*, 1971), Americans (Smith *et al.*, 1971) and British populations (Brown, 1955; Loraine and Bell, 1963). In the Swedish study, the mean levels of the pre-ovulatory peak in 34 cycles was 41·2±29·3 μg./24 hours and the luteal maximum was 23±2·5 μg./24 hours. In the British

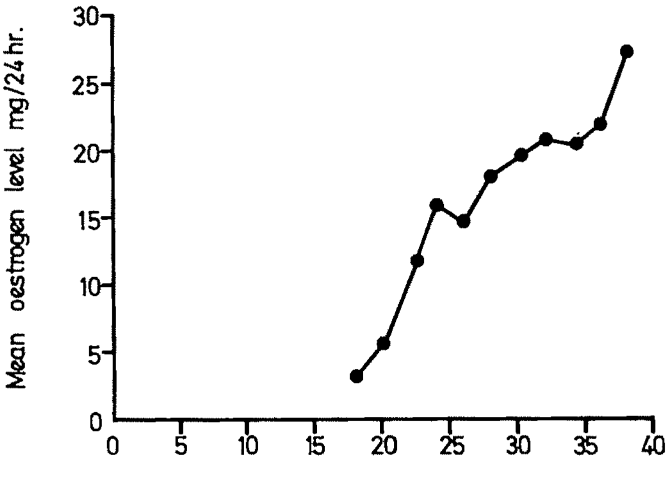

FIG. 3

Total urinary oestrogen levels during normal pregnancy in 76 subjects.

study of 15 cycles by Loraine and Bell (1963) the mean pre-ovulation peak was 48 μg./24 hours while the mean luteal maximum was 33 μg./24 hours. In the present study total oestrogen excretion during the 1st and 2nd days of the cycle was somewhat higher than that reported by these authors. Although our values for oestrogen excretion during the pre-ovulatory phase agreed with those of previous workers in other communities, the values for the luteal phase excretion were much higher than those previously reported. Indeed in 22 of the 23 cycles studied in Group 2, the luteal maximum oestrogen excretion was consistently found to be higher than the pre-ovulatory maximum. The possible significance of this observation in relation to follicular development and corpus luteum function in this environment is not clear. However, it must be observed that the non-pregnant subjects used in this study came from a population with a dizygotic twinning rate of 1 in 20 (Nylander, 1970), and it has been suggested that such high twinning rate may be due to a high output of pituitary FSH (Nylander, 1973). The relationship between the gonadotrophin secretion and ovarian function in this population deserves further study.

The pattern of oestrogen excretion we observed during pregnancy closely followed those reported in some other communities in early pregnancy (Taylor *et al.*, 1961; Coyle and Brown, 1963). However, the values reported by Boulle (1968) for African and Indian populations in South Africa were much higher than ours. Also, we did not observe the characteristic rapid rise in oestrogen excretion which is usually seen in late pregnancy although all ended in the normal delivery of a single fetus.

ACKNOWLEDGEMENTS

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# SCANNING ELECTRON MICROSCOPY OF NORMAL AND ABNORMAL EXFOLIATED CERVICAL SQUAMOUS CELLS

BY

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## Summary

The surface ultrastructure of normal, malignant and dyskaryotic exfoliated cervical squamous cells was examined with the Scanning Electron Microscope (SEM). Normal and malignant cells were found to have very similar surfaces to those seen on similar cells in a biopsy specimen. Dyskaryotic cells, however, showed a large range of surface features and dyskaryotic cells that looked identical at light microscopy could be subdivided into three groups by examining their ultrastructural features.

THE Scanning Electron Microscope (SEM) appearances of normal squamous and columnar epithelium of the cervix and also of the epithelium from areas of metaplasia and from carcinoma *in situ* have been described (Williams *et al.*, 1971; Jordan and Williams, 1971; Williams *et al.*, 1973*a* and *b*). A recent study by Murphy *et al.* (1974) showed that the SEM appearances of carcinoma *in situ* were reliable and reproducible in a large number of patients. The SEM has also been used to study individual exfoliated cells (Friedlander, 1969; Murphy *et al.*, 1973*a* and *b*; Llanes *et al.*, 1973). Friedlander (1969) noted differences between benign and malignant cells but did not make any effort to identify the same cell by conventional techniques. Llanes *et al.* (1973) confined their study to normal squamous cells. They reported a difference in surface ultrastructure between basal cells and superficial squamous cells, the former being covered by microvilli and the latter being covered by a system of branching microridges. Murphy *et al.* (1973*a* and *b*), in their study of normal and abnormal cervical cells, reported on methods for identifying the same cell prior to SEM study. They showed that surface details were diminished in exfoliated cells and attributed this to the fact that the cells were damaged during the process of air drying. Recently, critical point drying techniques (Boyde and Wood, 1969) were introduced in their study, resulting in a marked improvement in preservation of surface structure. These techniques also allowed cells to be stained and identified by light microscopy prior to SEM study.

The present study reports the findings at SEM of normal, dyskaryotic and malignant looking cells from the human cervix.

## METHODS

All cell samples were obtained from patients attending either the colposcopy clinic for the investigation of abnormal cervical cytology, or the general gynaecological clinic but with known normal cervical cytology. Two basic techniques were used to obtain cells.

*Direct scrape technique*

The cervix was scraped with an Ayre's spatula as when taking a routine smear. The cells were smeared gently onto a graticuled slide (S55 Finder, Graticules Limited, Tunbridge, Kent, U.K.), fixed in 2·5 per cent gluteraldehyde in cacodylate buffered sucrose, and stained with haematoxylin. Counter-staining was not used because sufficient cellular detail could be observed without it and because of a general reluctance to use several reagents prior to SEM examination. During the examination the slide was kept moist with distilled water. Once cells of interest were located the relevant area of the glass slide was cut out. The small square so obtained (1 × 1 cm.) was then dehydrated in graded ethanol solutions of 25 per cent, 50 per cent, 75 per cent and 100 per cent for five minutes at each stage. The specimen was then transferred from 100 per cent ethanol to graded amyl acetate ethanol mixtures of 25 per cent, 50 per cent, 75 per cent and 100 per cent amyl acetate for five minutes at each stage and was then placed in the critical point drying apparatus. The critical point drying was carried out from amyl acetate and liquid carbon dioxide.

*Membrane filter technique (adapted from Evans* et al., *1969)*

The cervix was swabbed gently with 0·85 per cent saline to remove excess mucus. A small piece of membrane filter (millipore filter; 5 × 9 mm., grade HA, 0·45 $\mu$m. pore-size) was placed on the cervix under colposcopic direction and left there for five to ten seconds. The filter was then peeled away from the cervix, stripping with it a layer of surface cells from the epithelium. The filter was placed immediately in fixative (2·5 per cent gluteraldehyde in cacodylate buffered sucrose) for one hour at 4 °C. As the filters were soluble in acetone, dehydration was carried out using graded ethanol solutions, and they were then dried by the critical point technique. The drying was carried out directly from ethanol as the filters tended to be soluble in amyl acetate. The dried specimens, cell side uppermost, were mounted on a stub with araldite and coated *in vacuo* with gold palladium alloy (about 20 nm.) before examination in the SEM. All specimens were examined in a stereoscan S4 (Cambridge Scientific Instrument Company) using an accelerating voltage of 20 kv. and a tilt angle of 45°.

## RESULTS

Of the 170 patients studied, 30 were known to have normal cervical cytology, 85 had malignant cells, whereas 55 had dyskaryotic cells in a smear. All 85 patients with malignant cells present were found to have carcinoma *in situ* at subsequent histological examination. The 55 patients with dyskaryosis have not had a biopsy and are being followed-up with cytology and colposcopy. The colposcopic lesion has been in each patient less severe than carcinoma *in situ*. The results from the various techniques are set out in Table I. The results were deemed technically satisfactory when sufficient surface detail was present to allow diagnosis based on criteria already established from SEM examination of

TABLE I

*Results of the two methods used to obtain cells for SEM examination*

| Methods of sampling | Patients with normal cytology | | Patients with malignant cells | | Patients with dyskaryotic cells | |
|---|---|---|---|---|---|---|
| | Number | No. of preparations technically satisfactory for SEM | Number | No. of preparations technically satisfactory for SEM | Number | No. of preparations technically satisfactory for SEM |
| Scrape | 20 | 19 | 45 | 40 | 55 | 55 |
| Membrane filter | 10 | 8 | 40 | 37 | – | – |

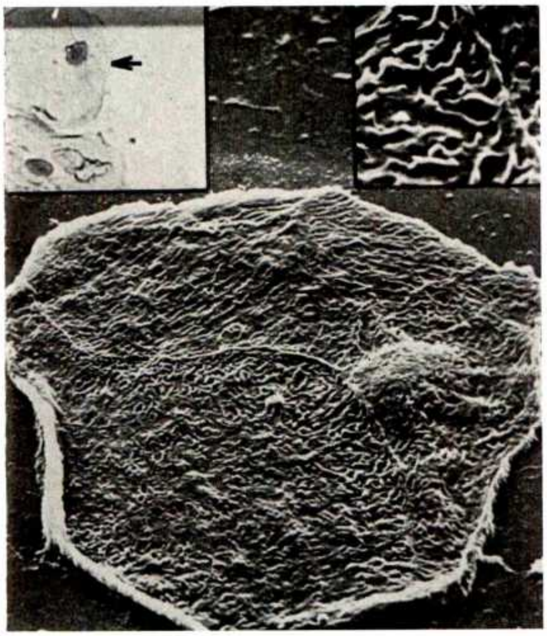

FIG. 1
Normal superficial squamous cell (× 5300). Inset on left shows same cell (arrowed) at light microscopy (× 250) Inset on right shows surface microridges (× 11000).

biopsy material (Williams *et al.*, 1971; Jordan and Williams, 1971; Williams *et al.*, 1973*a* and *b*).

*Cell morphology*

*Normal cells.* Normal superficial squamous cells showed essentially the same features at SEM examination as similar cells in biopsy material (Fig. 1). The cells showed the characteristic features of normal squamous epithelium at both low and high magnifications. The surface was composed of microridges, and the preservation of surface features of this cell compared most favourably with that of similar cells from biopsy preparations. A feature of exfoliated normal squamous cells was the presence of bars on the surface. These bars were considered to be the markings of the borders of the overlapping cells that had been present prior to the cells being removed from the cervix. Figure 2 shows a colpophotograph of a normal cervix with a sampling membrane filter in position, and in Figure 3 a sheet of normal squamous cells from this membrane filter can be seen. The whole appearance of this sheet of squamous cells was most similar to superficial squamous epithelium seen at biopsy and at higher magnification

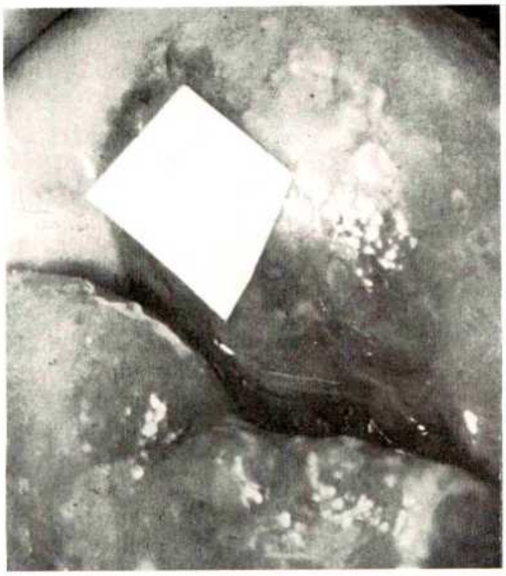

FIG. 2
Colpophotograph with sampling membrane filter in position (× 2).

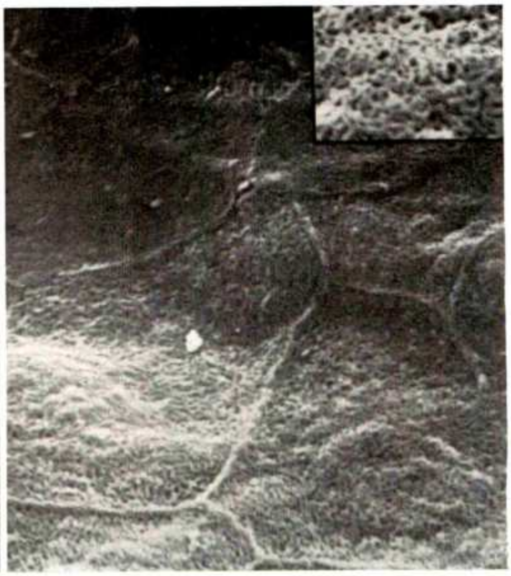

FIG. 3
Sheet of normal squamous cells on filter from Figure 2 (× 2000). Inset shows normal surface microridges (× 10000).

characteristic microridges were obvious. Six of the patients with normal cytology who were postmenopausal had basal and parabasal cells in their cervical scrape (Fig. 4). Basal cells were rounded and smaller than normal superficial squamous cells and the surface was found to be covered with a microridge structure. The ridges were not as straight as those in more superficial cells and were often in a circular type of arrangement. Surface features resembling those seen in the abnormal cells were not observed in basal or parabasal cells from this group.

*Malignant cells.* Cells were considered to be abnormal on the basis of their shape, size, their general architecture and surface features. Only with the membrane filter was the relationship of cells to each other of importance in making a diagnosis at SEM. A group of cells considered to be malignant smeared onto a marked glass slide and stained with haematoxylin and one of these cells at SEM study can be seen in Figure 5. The cells were rounder and smaller than normal squamous cells and their surfaces were covered with closely packed microvilli. A sheet of abnormal looking cells is shown on a membrane filter preparation from an area of carcinoma *in situ* (Fig. 6). The cells were small, bizarre in their relationship with each other when compared with normal cells on a membrane filter, and their surfaces showed microvilli. The appearances of these cells correlated with those described for carcinoma *in situ* when studied in a biopsy specimen (Williams *et al.*, 1973*a* and *b*).

*Dyskaryotic cells.* Of the 55 patients studied with dyskaryotic cells, 40 patients had mild dyskaryosis only, while 15 had a mixture of mildly and severely dyskaryotic cells on the smear. From these patients 138 cells were studied; 102 with mild and 36 with severe dyskaryosis. The surface features obtained from these cells are set out in Table II. Figure 7 shows a severely dyskaryotic stained cell at light microscopy and the same cell at SEM examination. The surface features of this cell were identical to those seen on malignant looking cells from the cervix. Another severely dyskaryotic stained cell at light microscopy and at SEM examination is seen in Figure 8. The surface features of this cell were considered normal by already described SEM characteristics. In Figure 9 a mildly dyskaryotic cell is seen at light microscopy, and at SEM study the surface features of this cell

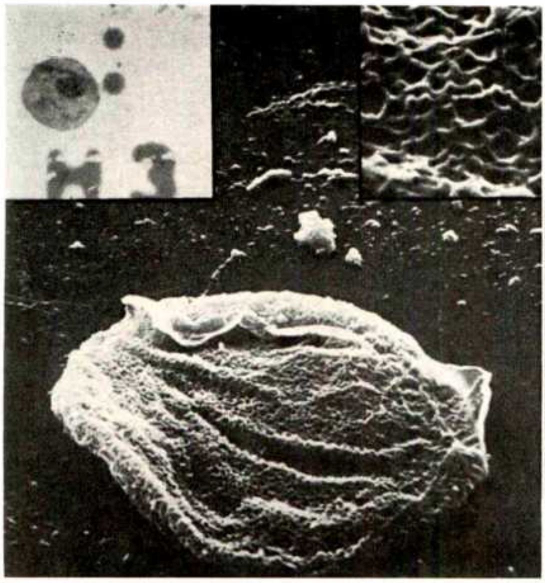

FIG. 4

Normal parabasal cell (× 1900). Inset on left shows same cell at light microscopy (× 250). Inset on right shows microridge surface structure (× 9500).

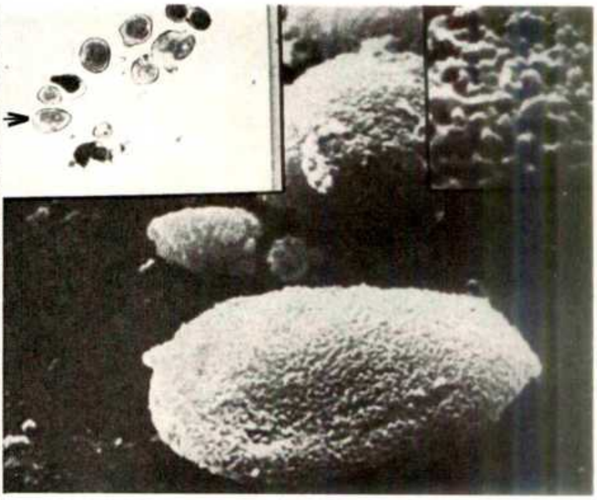

FIG. 5

Malignant cell (× 2800). Inset on left shows same cell (arrowed) at light microscopy (× 250). Inset on right shows surface microvilli (× 14000).

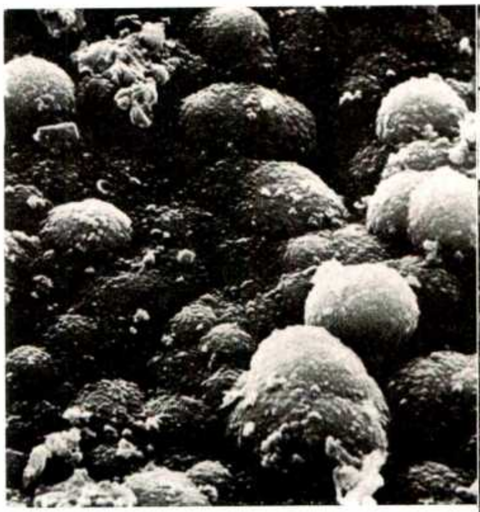

FIG. 6

Sheet of abnormal cells on membrane filter preparation from an area of carcinoma *in situ* (×2000).

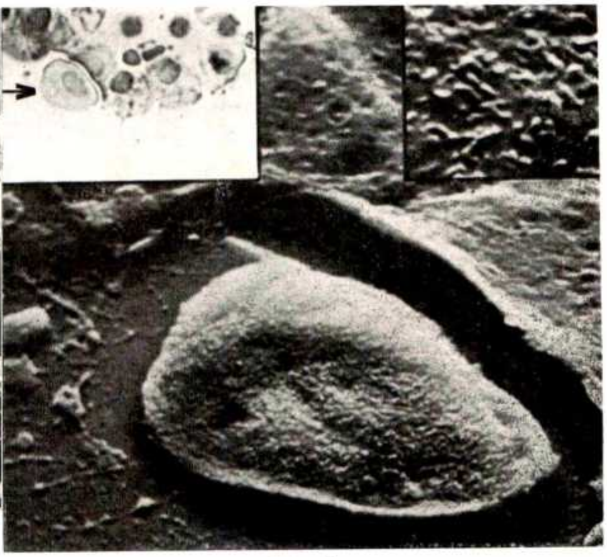

FIG. 7

Severely dyskaryotic cell (×2000). Inset on left shows same cell (arrowed) at light microscopy (×250). Inset on right shows surface microvilli (×10500).

TABLE II

*Dyskaryotic cells examined with the SEM*

*Breakdown of patients*

55 patients — 40 had mildly dyskaryotic cells only
55 patients — 15 had a mixture of mildly and severely dyskaryotic cells

*Cells studied*

138 — 102 mild dyskaryosis — 47 abnormal surface features
138 — 102 mild dyskaryosis — 28 intermediate surface features
138 — 102 mild dyskaryosis — 27 normal surface features
138 — 36 severe dyskaryosis — 28 abnormal surface features
138 — 36 severe dyskaryosis — 5 intermediate surface features
138 — 36 severe dyskaryosis — 3 normal surface features

are those ordinarily associated with malignancy. Figure 10 shows another mildly dyskaryotic cell at light microscopy, and the same cell examined at SEM. The surface of this cell was considered normal. Twenty-seven such cells were studied from 10 patients. In 28 cell preparations from seven different patients, mildly dyskaryotic cells were seen (Figure 11 shows one of these cells) and when these cells were viewed with the SEM, the surface showed an appearance which was intermediate between microridges and microvilli. The features of two mildly dyskaryotic

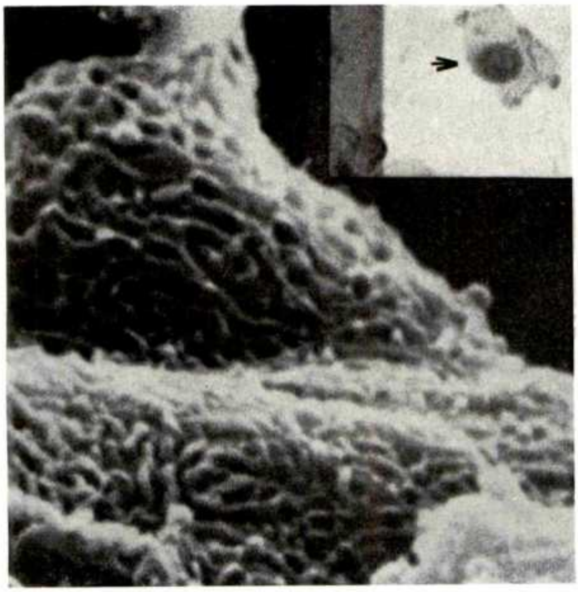

FIG. 8

Severely dyskaryotic cell showing normal surface microridges (× 9000). Inset on right shows same cell (arrowed) at light microscopy) (× 250).

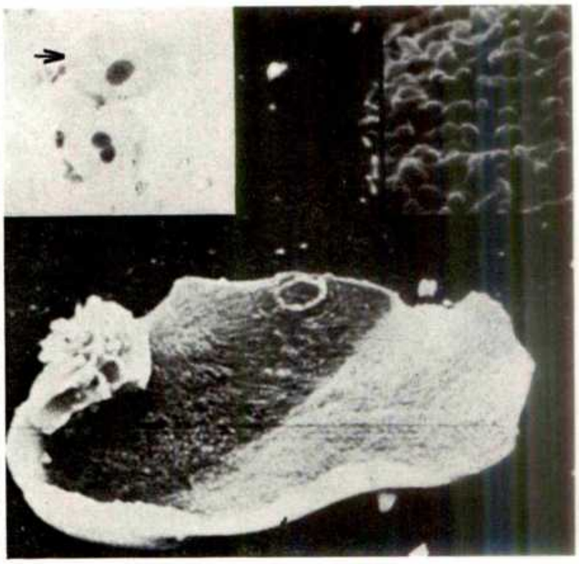

FIG. 9

Mildly dyskaryotic cell (× 1100). Inset on left shows same cell (arrowed) at light microscopy (× 250). Inset on right shows surface microvilli (× 7500).

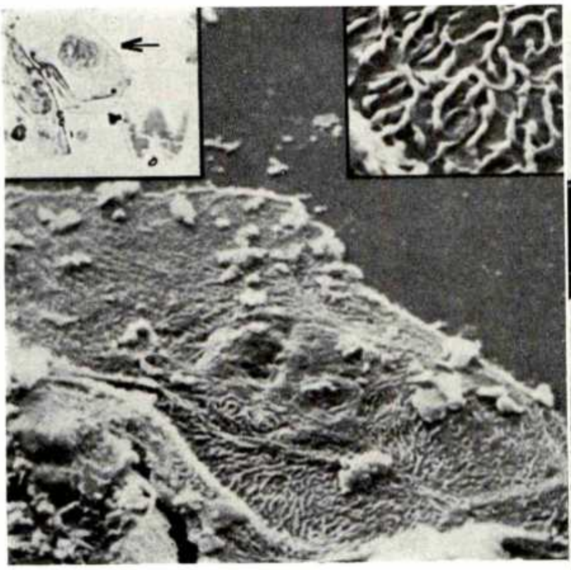

FIG. 10

Mildly dyskaryotic cell (× 1300). Inset on left shows same cell (arrowed) at light microscopy (× 250). Inset on right shows surface microridges (× 6400).

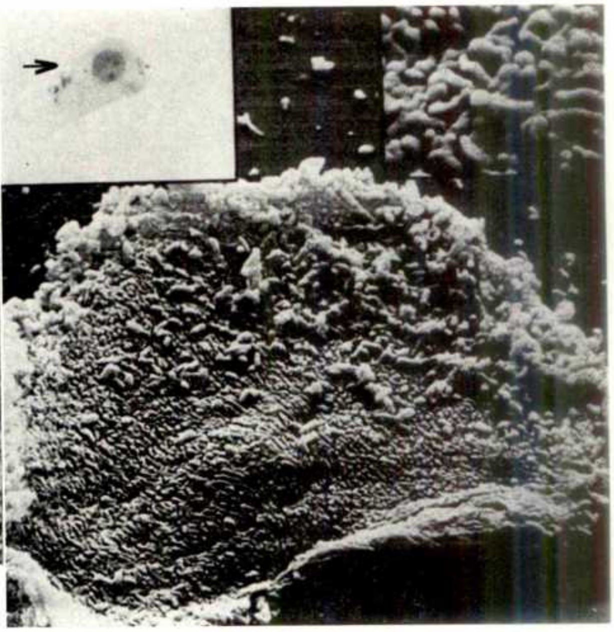

FIG. 11

Mildly dyskaryotic cell (× 1500). Inset on left shows same cell (arrowed) at light microscopy (× 250). Inset on right shows intermediate type surface features (× 7500).

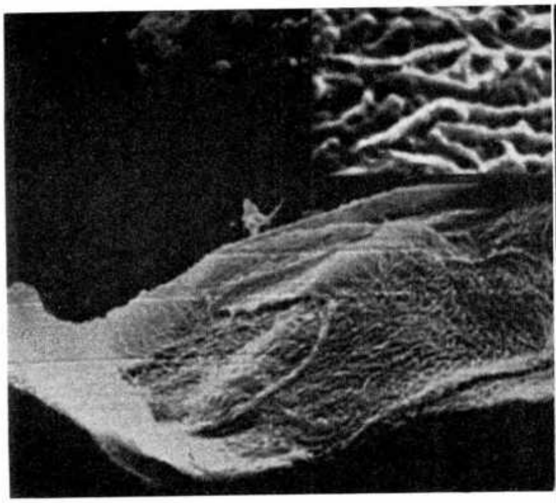

FIG. 12
Mildly dyskaryotic cell at SEM examination (× 2200). Inset shows its normal surface microridges (× 11000).

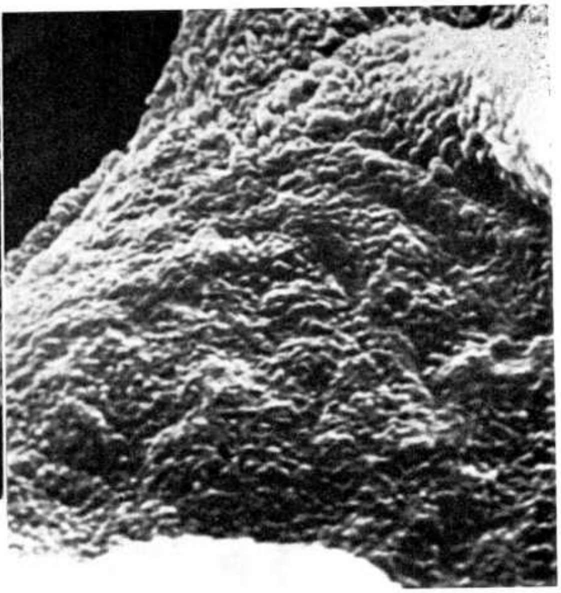

FIG. 13
Mildly dyskaryotic cell showing abnormal surface features with microvilli (× 5500).

cells from the same smear are seen in Figures 12 and 13; one cell has normal surface features (Fig. 12) while the other does not (Fig. 13).

## DISCUSSION

The application of the SEM to the study of individual exfoliated cells present difficulties greater than when similar cells are studied in a biopsy specimen. As air drying causes such a great loss in surface features, all specimens of individual cells should be dried by the critical point method. Both techniques for collecting cells provided satisfactory results. The smear technique produced adequate numbers of easily recognised cells. The disadvantage, however, of this technique is that at the moment it is time-consuming as cells first have to be examined and identified by light microscopy and then again by SEM. With the membrane filter technique the cells were not examined by light microscopy, but were taken from an area on the cervix known colposcopically to be normal or abnormal. The membrane filter technique has one major advantage over the other methods in that it strips off a sheet of cells which bear a relationship to each other similar to that seen *in vivo*. This relationship is important in diagnosing epithelial abnormalities. Both benign and malignant cells essentially showed the same features as those seen on similar cells from biopsy specimens, except that in malignant cells the quality of the microvillous surface pattern was never as good as that seen from biopsy specimens. In general, cells that were severely dyskaryotic showed patterns very similar to those seen on malignant cells. The mildly dyskaryotic cells often showed quite different surface characteristics and mildly dyskaryotic cells can clearly be sub-grouped on the basis of their surface architecture. It could be that the cells with the abnormal surface features could be more likely to become malignant. A long term prospective study to elucidate this matter is in progress.

## ACKNOWLEDGEMENTS

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# FACTORS INFLUENCING THE PREGNANCY AND COMPLICATION RATES WITH HUMAN MENOPAUSAL GONADOTROPHIN THERAPY

BY

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## Summary

We report the results obtained with a standard system of gonadotrophin therapy. Seventy-seven consecutive patients were given 322 treatment cycles. Thirty-seven patients (48 per cent) conceived, six of them on two occasions, making 43 pregnancies of which 31·6 per cent were multiple. Five per cent of all treatment cycles were complicated by mild, and 0·62 per cent by severe hyperstimulation. The factors involved in achieving a satisfactory pregnancy rate whilst avoiding complications are discussed. Most complications occurred during the first cycle in which the rise in oestrogen excretion suggested follicular development and human chorionic gonadotrophin (HCG) was given (the "first effective" treatment cycle). In such cycles the risk of hyperstimulation rose sharply when the day 6 urinary total oestrogen level was above 150 μg. per 24 hours and the multiple pregnancy rate was increased by a large dose of HCG.

Gonadotrophin therapy is complex to manage for both the clinician and patient and treatment may be complicated by multiple pregnancy or hyperstimulation, mild or severe. In mild cases there is ovarian enlargement with either abdominal pain and/or distension but the condition resolves spontaneously. In severe cases there is ovarian enlargement, ascites, occasionally a pleural effusion, and sometimes secondary haemodynamic disturbance.

This paper analyses our results with human menopausal gonadotrophin therapy and is particularly concerned with the need to achieve a satisfactory pregnancy rate with a minimal incidence of complications.

## Patients and Methods

Seventy-seven consecutive patients with anovulation as the only known cause of their sterility were included in the series. One had primary and 32 had secondary amenorrhoea; 20 had regular and 19 irregular anovulatory cycles and five had the polycystic ovary syndrome. Their ages ranged from 23 to 40 years. Sixty-five patients had primary and 12 secondary infertility, ranging from one to 10 years duration, the average being 3·3 years.

All except seven patients had previously been treated with either clomiphene or, less commonly, tamoxifen (Williamson and Ellis, 1973). Each of the remaining seven patients, who were treated with gonadotrophins only, had basal 24-hour urinary total oestrogen levels of 8 μg. or less. According to basal temperature records, 32 of those patients previously treated unsuccessfully with anti-oestrogens had apparently ovulated at least once but none had conceived.

We used human menopausal gonadotrophin (HMG) prepared in ampoules containing 75 IU of follicle stimulating hormone (FSH) with 75 IU of luteinizing hormone (LH). The HMG was given in three equally divided doses on days 1, 3 and 5 and human chorionic gonadotrophin (HCG) was given on day 8, as described by Butler (1969). The response to treatment was monitored by measuring the 24-hour urinary total oestrogen output by the method of Brown *et al.* (1968) on days—2, 4, and 6.

Patients with amenorrhoea started with five ampoules of HMG on each treatment day and those with cyclical bleeding were given three ampoules. A 5000 IU dose of HCG was given on day 8. In subsequent cycles, the HCG dose on day 8 was increased or a further 5000 IU dose was given on day 15 if the oestrogen response (measured as the 24-hour urinary output on days 4 and 6) suggested good follicular development and the basal body temperature chart did not appear to be biphasic.

Seven to eight patients started a treatment cycle each week so that approximately 40 patients attended our clinic for HMG therapy at any one time.

## RESULTS

Seventy-seven women were given 322 treatment cycles, using 6269 ampoules of HMG. In 51 cycles the HCG was withheld as there had been no response or an excessive and potentially dangerous response to the HMG. Thirty-seven patients (48 per cent) conceived and between them had 43 pregnancies. Five pregnancies ended in abortion (including one late abortion of twins). In addition, there were 26 singleton births, eight sets of twins, three sets of triplets, and one set of quintuplets. There were thus 12 multiple pregnancies amongst 38 which lasted more than 28 weeks, so that the risk of having more than one viable baby was 31·6 per cent or approximately one in three whilst the risk of having more than two viable babies was 1 in 9·5 (or approximately 1 in $3^2$). There were 16 mild hyperstimulations (5 per cent of all treatment cycles) in 11 patients and two severe episodes (0·62 per cent of all treatment cycles).

The 38 pregnancies lasting more than 28 weeks resulted in 56 livebirths and no stillbirths. However, one singleton pregnancy ended in spontaneous labour at 28 weeks and the baby, a boy weighing 1·13 kg. died during the early neonatal period. The quintuplet pregnancy also ended in premature labour at 30 weeks and all five babies died during the neonatal period. Altogether 50 babies survived of whom four had congenital abnormalities; two had an umbilical hernia, one a small occipital meningocele which was surgically corrected and one the Pierre-Robin syndrome (micrognathia, cleft palate, and glossoptosis).

### *Factors affecting pregnancy rate*

Patients with secondary amenorrhoea and those with the polycystic ovary syndrome were treated with the most success (Table I). The pre-treatment urinary oestrogen level was inversely related to the pregnancy rate. Of 17 patients with urinary total oestrogens of less than 7 μg. per 24 hours, 12 (71 per cent) conceived 15 times. In contrast, of 19 women with 24-hour urinary total oestrogens above 20 μg. only 7 (37 per cent) conceived having 7 pregnancies in all. In the remaining 41 women with oestrogen levels between 7 and 20 μg. per 24 hours, 18 (44 per cent) conceived 21 times. However, the lower the pre-treatment oestrogen

TABLE I

*Pregnancies in diagnostic groups*

| Diagnosis | No. of patients | No. of treatment cycles | Pregnancies |
|---|---|---|---|
| Primary amenorrhoea | 1 | 3 | |
| Secondary amenorrhoea | 32 | 144 | 24 (19) |
| Irregular anovulatory cycles | 19 | 78 | 7 (7) |
| Regular anovulatory cycles | 20 | 84 | 8 (8) |
| Polycystic ovary syndrome | 5 | 13 | 4 (3) |
| Total | 77 | 322 | 43 (37) |

(No. of patients in brackets)

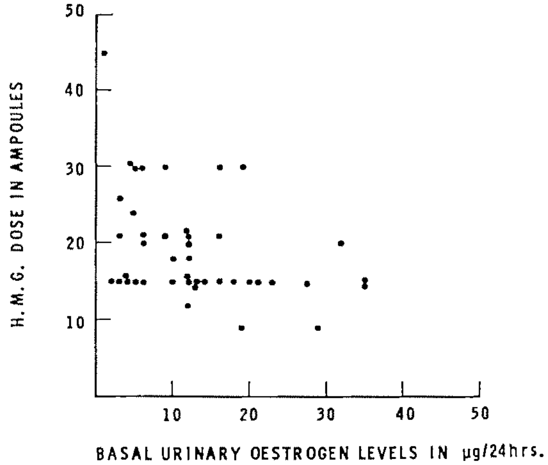

FIG. 1

HMG dosage (in ampoules) used in pregnancy cycles at each basal urinary oestrogen level.

level the higher the dose of HMG that was required. Figure 1 illustrates the scatter of HMG dosage in the treatment cycles resulting in pregnancy in relation to the basal urinary oestrogen levels. It can also be seen (Fig. 1) that only one patient conceived after being given more than 30 ampoules of HMG although nine patients were given a higher dose in a total of 20 cycles.

The 24-hour urinary oestrogen level on day 6 bore some relationship to the pregnancy rate which increased a little with higher oestrogen values (Table II). The HCG dose showed a more marked relationship to the pregnancy rate (Table III); large single doses or more than one HCG injection increased the pregnancy rate.

TABLE II

*Number of pregnancies in relation to the oestrogen level on day 6*

| Urinary oestrogen level ($\mu$g./24 hours) | Number of cycles in which HCG was given | Number pregnant | Percentage of treatment cycles resulting in pregnancy |
|---|---|---|---|
| less than 50 | 52 | 7 | 13·5 |
| 50 to 100 | 93 | 13 | 14·0 |
| 101 to 150 | 47 | 8 | 17·0 |
| 151 to 250 | 49 | 8 | 16·3 |
| more than 250 | 30 | 6* | 20·0 |

* There was one further pregnancy when HCG was not given.

TABLE III

*Number of pregnancies at each HCG dose*

| HCG dose (in 1000 IU) | Number of cycles | Number of preg-nancies | Percentage of treatment cycles followed by pregnancy |
|---|---|---|---|
| 5 | 115 | 13 | 11·3 |
| 10 | 104 | 16 | 15·4 |
| 15 | 7 | 3 | 42·9 |
| Two HCG injections | 45 | 10 | 22·2 |

There was a further pregnancy when HCG was not given making the total number of pregnancies 43.

Hyperstimulation apparently increased the prospect of pregnancy. Six (33 per cent) of 18 treatment cycles complicated by hyperstimulation resulted in pregnancy whilst only 37 pregnancies (12 per cent) followed 304 uncomplicated treatment cycles.

Figure 2 shows the distribution of the number of treatment cycles per patient. No conception occurred after eight treatment cycles and only 4 of 23 patients given more than four treatment cycles conceived.

The response to previous anti-oestrogen therapy did not influence the pregnancy rate. Of 70 patients treated primarily with anti-oestrogens, we know the response in 67. According to basal body temperature records there was no evidence of ovulation in 35 patients whilst 32 apparently ovulated on at least one occasion; in both groups just under 50 per cent (17 and 15 patients respectively) conceived following HMG therapy.

### *Factors affecting the multiple pregnancy rate*

There was a total of 13 multiple pregnancies. Larger doses of HCG increased the multiple pregnancy rate. When 5000 IU were given there were only two multiple pregnancies in ten compared with 10 in 28 when a larger dose or a second injection of HCG was given. There was also one twin pregnancy after a treatment cycle in which no HCG was given. This effect of HCG was more marked in the first cycle in which the

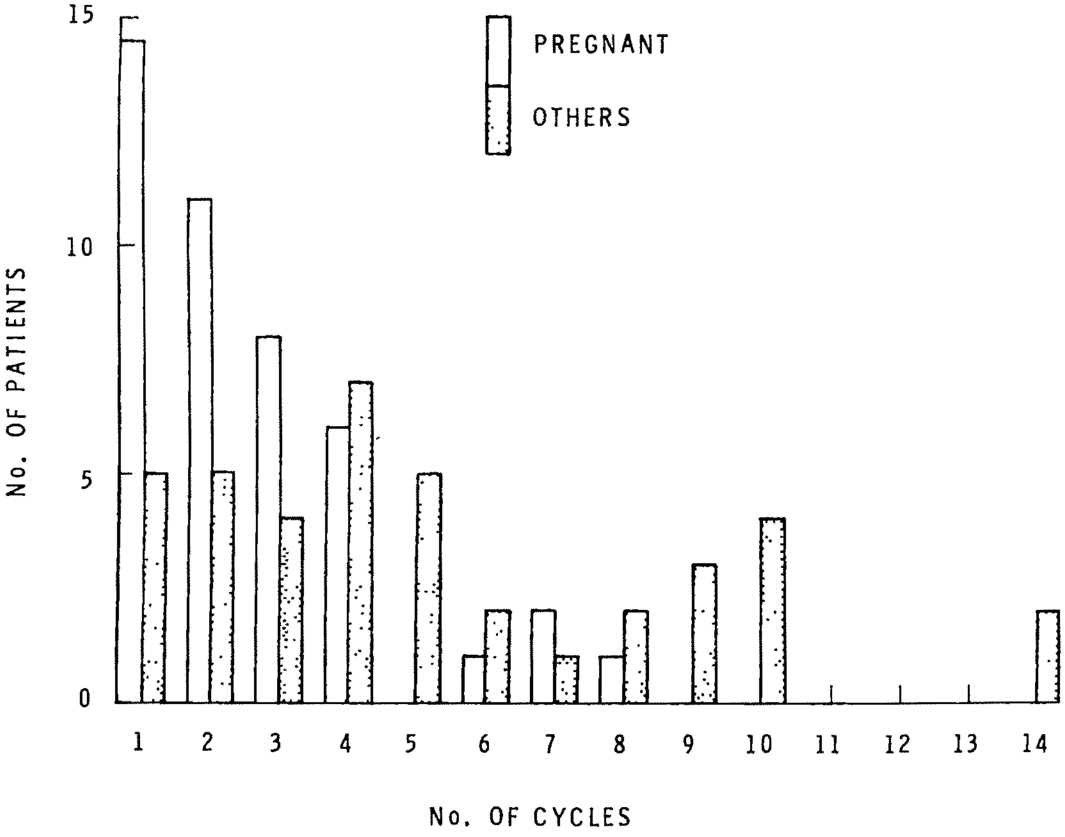

FIG. 2
Number of treatment cycles given to all patients (six patients conceived twice)

rise in oestrogen excretion suggested follicular development and HCG was given (the "first effective" treatment cycle). Four out of five pregnancies were multiple when 10 000 IU of HCG was given in such cycles compared with two multiple pregnancies out of seven following 5000 IU of HCG. There were no multiple pregnancies in subsequent cycles when 5000 IU of HCG was given. The incidence of multiple pregnancy was also related to the 24-hour urinary oestrogen level on day 6 for it was 50 per cent when the value was above 150 μg. compared to 24 per cent with values below that level.

Of six pregnancies which occurred during hyperstimulation cycles, three were multiple, including two with more than two babies, whereas in 37 pregnancies which occurred during uncomplicated treatment cycles only ten were multiple. However, the one pregnancy occurring after a treatment cycle complicated by severe hyperstimulation was a singleton.

The diagnostic category had little influence on the multiple pregnancy rate. There were eight multiple pregnancies (38 per cent) amongst 21 pregnancies in patients with secondary amenorrhoea, three (38 per cent) amongst eight pregnancies in patients with regular cyclical bleeding and two (50 per cent) amongst four pregnancies in patients with the polycystic ovary syndrome. Likewise, the pre-treatment 24-hour urinary oestrogen level had no significant influence on the multiple pregnancy rate. In those with a 24-hour total oestrogen level less than 7 μg., four (31 per cent) out of 13 pregnancies were multiple compared with three multiple pregnancies (50 per cent) out of six when the value was greater than 20 μg. per 24 hours. With values between 7 and 20 $\mu$g. per 24 hours 6 out of 20 pregnancies (30 per cent) were multiple.

Of the 13 multiple pregnancies, eight were conceived during "first effective" treatment cycles. Also, three of the four pregnancies resulting in three or more fetuses occurred in one of these cycles.

### *Factors affecting the hyperstimulation rate*

The urinary oestrogen level on day 6 markedly influenced the hyperstimulation rate. With values above 150 $\mu$g. per 24 hours there were 13 mild (16 per cent) and two severe (3 per cent) hyperstimulations amongst 79 treatment cycles in which HCG was given compared with two mild hyperstimulations amongst 192 cycles (1 per cent) with values below this level. This effect was even more marked in "first effective" treatment cycles (Table IV). Both the severe hyperstimulations occurred in such cycles in which the day 6 urinary total oestrogen level was above 250 $\mu$g. per 24 hours. One was in a patient with secondary amenorrhoea (reported by Tyack and Ellis, 1971) while the other was in a patient with irregular anovulatory cycles who conceived during the hyperstimulation cycle. Both patients recovered completely and the pregnancy in the second patient progressed normally to term with delivery of a healthy boy weighing 2·67 kg.

The pre-treatment oestrogen level also had a marked influence. When the 24-hour total oestrogen level was less than 7 $\mu$g. there was one mild hyperstimulation amongst 62 cycles (1·6 per cent) in which HCG was given compared with six mild (9·4 per cent) and one severe (1·6 per cent) hyperstimulations amongst 64 cycles when the 24-hour urinary oestrogen level was above 20 $\mu$g. With values between 7 and 20 $\mu$g. per 24 hours there were eight mild (5 per cent) and one severe (0·62 per cent) hyperstimulations in 161 treatment cycles. In the last category there was also one instance of mild hyperstimulation in a cycle in which HCG was not given.

TABLE IV

*Hyperstimulations in relation to the oestrogen level on day 6 in first treatment cycles in which HCG was given*

| Urinary oestrogen level on day 6 ($\mu$g./24 hours) | Number of cycles | Hyperstimulations | |
|---|---|---|---|
| | | Mild | Severe |
| less than 50 | 14 | | |
| 50 to 100 | 24 | | |
| 101 to 150 | 14 | 1 (7·1%) | |
| 151 to 250 | 16 | 5 (31·3%) | |
| more than 250 | 8 | 4* (50%) | 2 (25%) |

* There was one further mild hyperstimulation in a first cycle in which HCG was not given.

The HCG dose had less influence on the hyperstimulation rate. When a single 5000 IU dose of HCG was given there were nine mild hyperstimulations and one severe hyperstimulation in 115 treatment cycles compared with four mild hyperstimulations and one severe hyperstimulation in 104 treatment cycles in which 10 000 IU of HCG was given as a single dose. However, if "first effective" cycles are considered there were eight mild hyperstimulations and one severe hyperstimulation in 63 treatment cycles with a 5000 IU dose of HCG compared with two mild hyperstimulations and one severe hyperstimulation in ten treatment cycles in which 10 000 IU of HCG were given.

The diagnostic category had little influence except that there were two mild hyperstimulations (15 per cent of treatment cycles in which HCG was given) in patients with the polycystic ovary syndrome.

Eleven of the 16 mild hyperstimulations and both severe hyperstimulations occurred during "first effective" treatment cycles. Indeed, with one exception, all hyperstimulations occurred in such cycles or in patients who had shown evidence of hyperstimulation in the "first effective" cycle. Even in the one exception a mild hyperstimulation had occurred in a first cycle in a previous course of treatment.

## COMMENTS

The results presented led us to a number of helpful conclusions about the management of patients having gonadotrophin therapy.

The most successful patient to treat is one with low pre-treatment oestrogen levels. The pregnancy rate is high and the incidence of hyperstimulation and multiple pregnancy is low. However, the pregnancy rate with any group is over 30 per cent. Patients who appear to ovulate with clomiphene therapy but do not conceive, are worth treating with gonadotrophins. Lunenfeld (1970) and Bettendorf (1970) both came to a similar conclusion.

There is a limit to the number of treatment cycles which can usefully be given. Over 90 per cent of the pregnancies in the present series were conceived with four or less treatment cycles. Also 30 ampoules of HMG per treatment cycle may be a useful ceiling dose, only one patient having conceived with a higher dose.

HCG should not be given in a "first effective" treatment cycle when the day 6 urinary oestrogen level rises to 150 $\mu$g. per 24 hours or above. The risk of hyperstimulation is too great and the pregnancy rate is not much improved over lower levels (Table II). Even in subsequent cycles one should be careful although it is sometimes necessary to take the risk of giving HCG to achieve pregnancy.

The results support the suggestion of Brown *et al.* (1969) that multiple pregnancy could be associated with a higher than necessary HCG dose. The minimum effective dose (which may well be less than 5000 IU) should be used in the "first effective" cycle. However, this will also reduce the number of pregnancies so the dose should be progressively increased in subsequent cycles to improve the pregnancy rate.

The "first effective" treatment cycle is the most important as the pregnancy and multiple pregnancy rates are higher than in any other cycle and the great majority of hyperstimulations occur in it.

### ACKNOWLEDGEMENTS

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# TUBAL PREGNANCY AFTER STERILIZATION

BY

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## Summary

Twelve cases of tubal pregnancy after sterilization operations are reported and the pathogenesis is discussed. Diagnosis is sometimes delayed because of the history of previous sterilization. It is paradoxical that the diagnosis of an intrauterine pregnancy after tubal sterilization is rarely in doubt yet the possibility of the fertilized ovum implanting in a tube known to have been damaged is sometimes overlooked. It is suggested that bilateral salpingectomy is the correct operative treatment for this condition.

## PATIENTS AND METHODS

HALF of the cases presented were obtained by reference to operating theatre registers at Dulwich Hospital from 1970 to 1973 inclusive, while the rest were brought to our notice by colleagues. Where either the original sterilization or the subsequent operation for ectopic pregnancy was performed elsewhere, we sought full information from the hospital concerned. Particular attention has been given to the following details: method and timing of sterilization, length of time which elapsed between the sterilization and the ensuing ectopic pregnancy, clinical presentation of the tubal pregnancy, diagnostic difficulties and final operative treatment.

## RESULTS

Fifty consecutive cases of ectopic pregnancy were operated upon at Dulwich Hospital during the years under consideration, and six of these patients had been sterilized prior to the occurrence of ectopic gestation.

The method and timing of sterilization in all twelve cases are shown in Table I. The heading "side-to-side" describes a method where the divided segments of Fallopian tube are religated and overlapped side to side.

Table II shows the clinical presentation of the ectopic pregnancies. Cases 6 and 7 occurred in the same patient with one year intervening. We have included the provisional diagnosis and the time interval between hospital admission and operative intervention as indications of the degree of diagnostic difficulty.

In seven cases implantation had occurred in the distal (fimbrial) portion of the tube while five were in the proximal (cornual) end.

## DISCUSSION

Tubal sterilization was a factor in 12 per cent of a consecutive series of fifty cases of tubal pregnancy, and so is deserving of greater attention than hitherto. The incidence of ectopic pregnancy following sterilization has been variously reported as two cases in 1049 puerperal sterilizations (Lu and Chan, 1967) and one in

TABLE I

*Method and time of sterilization*

| | Pomeroy | Side-to-side | Dia-thermy and division | Dia-thermy |
|---|---|---|---|---|
| Puerperal | 5 | – | – | – |
| Post-abortal | 2 | 1 | 1 | 1 |
| Interval | 1 | – | – | – |

833 mixed sterilizations (Barglow and Eisner, 1966). When cases of failed sterilization were considered, however, it was found that at least one-third of subsequent pregnancies are ectopic (Wacek and Glatthaar, 1965).

All but two of the sterilizations in our series were puerperal or postabortal which may reflect the higher failure rate of the operation at these times (Murdoch, 1969). Most of the popular methods of sterilization have contributed to this series.

The clinical presentation of our patients has followed the expected pattern of any ectopic gestation, the only constant features being abdominal pain, and abdominal and pelvic tenderness. In six of the patients there was an interval of more than twelve hours between hospital admission and operative treatment, and in eight the provisional diagnosis was incorrect. Perusal of the case notes makes it clear that delay and misdiagnosis were in some instances encouraged by the knowledge of the prior sterilization, but the diagnosis of ectopic pregnancy should be seriously considered in all previously sterilized patients who present with lower abdominal pain and tenderness. The main danger lies in diagnosing a condition which does not require immediate laparotomy.

The interval between sterilization and the presentation of the ectopic pregnancy varied from four months to nine years. None of our patients was of the variety described by Amin (1974) where tubal implantation appears to have taken place shortly before tubal diathermy. In seven of our 12 cases the site of implantation was the segment of the tube on the fimbrial side of the operative site. We postulate that recanalization (Kalchman and Meltzer, 1966) is the likely mechanism here, with the production of a narrow lumen, sufficient to allow the passage of spermatozoa but not of a fertilized ovum. The other five patients showed implantation proximal to the operative site, suggesting the formation of a tuboperitoneal fistula (Simpson *et al.*, 1961), which permitted the passage of an ovum into the tube where it was subsequently fertilized. Why it should then fail to reach the uterine cavity is uncertain, but a possible mechanism is tubal kinking due to adhesions.

Drake (1966) reported a case of two ectopic

TABLE II

*Clinical presentation of ectopic pregnancies after sterilization*

| Case No. | Sterilization to ectopic pregnancy interval | Amenorrhoea | Pain | Vaginal bleeding | Mass | Haemoglobin (g. per 100 ml.) | Provisional diagnosis | Admission to operation interval |
|---|---|---|---|---|---|---|---|---|
| 1 | 14 months | 0 | + | 0 | 0 | 10·6 | Intestinal obstruction | 4 hours |
| 2 | 13 months | 0 | + | 0 | 0 | 11·4 | Ectopic pregnancy | <4 hours |
| 3 | 4 years | 0 | + | + | 0 | 12→8 | Salpingitis | 24 hours |
| 4 | 2 years | 8 weeks | + | 0 | 0 | 9·2 | Ovarian cyst | 15 hours |
| 5 | 16 months | 0 | + | + | + | — | Appendicitis | <4 hours |
| 6* | 8 years | 0 | + | 0 | + | 10→5 | Ectopic pregnancy | 16 hours |
| 7* | 9 years | 0 | + | 0 | 0 | 10·6 | Ovarian cyst | 6 hours |
| 8 | 18 months | 0 | + | + | 0 | 13·2 | Salpingitis | 6 days |
| 9 | 4 months | 6 weeks | + | 0 | 0 | 9·3 | Appendicitis | 24 hours |
| 10 | 15 months | 0 | + | 0 | 0 | 11·2 | Salpingitis | 5 days |
| 11 | 2 years | 6 weeks | + | 0 | 0 | — | Ectopic pregnancy | <4 hours |
| 12 | 3 years | 8 weeks | + | + | + | 10·7 | Ectopic pregnancy | 6 hours |

* Same patient.

pregnancies in the same patient subsequent to the Pomeroy method of sterilization, and a similar case is included in our series. Removal of the opposite tube should therefore be performed when operating for tubal pregnancy in a patient who has undergone a previous sterilization, though diathermy coagulation of the cornual end may be an acceptable alternative. Unilateral salpingectomy was performed in eight, and bilateral salpingectomy in the other four of our patients.

## ACKNOWLEDGEMENTS

We thank the Consultant Gynaecological staff at Dulwich Hospital for permission to report patients under their care, and also Mr. D. J. Reid, Consultant Surgeon at Cuckfield Hospital, who provided access to two of the patients.

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# TREATMENT OF STRESS INCONTINENCE BY A FIBRIN BIOPLAST

BY

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## Summary

A new simple method of treating urinary stress incontinence is to fix a fibrin implant vaginally to the bladder neck and upper urethra. The support provided by the implant is maintained after its resorption by the fibrous tissue which replaces it. The two-year cure rate in 85 patients was 93 per cent.

FOR the cure of stress incontinence a metal or synthetic prosthesis has been placed under the vesicourethral junction. Goldberger and Davids (1947) used tantalum, Pecherstorfer (1953) and Palmrich and Froewis (1954) plexiglass, Marsalek (1955) silon, and Kraatz (1954) perlon net. However, these prostheses, fixed under the urethra or to the lower edge of the pubic arch, were often expelled.

Chassar Moir (1968) introduced a hammock operation, fixing a teflon sling to the vesicourethral junction and then to the fascia of the anterior abdominal wall. The sling gives permanent support but the operation is technically difficult.

We have introduced a simple and safe method of curing stress incontinence by a fibrin implant.

## METHOD AND RESULTS

A kidney-shaped 16×18×3 mm. Bioplast prosthesis is used, The Bioplast is made of human fibrin (Gerendás, 1959). It has a silicone-rubber like consistency. The unique features of this material are that it is completely free from undesirable reaction, is absorbable, and induces a marked fibrous tissue proliferation with insignificant shrinking and minimal scar formation. Histological analysis showed minimal tissue reaction (Kovács *et al.*, 1965).

Under local anaesthesia a midline incision was made in the anterior vaginal wall from urethra to cervix. The lateral flaps were dissected widely on both sides to expose and mobilize the urethra, the vesicourethral junction and bladder. The last was also separated from the cervix. The wide dissection was essential to prevent excessive tension over the implant. The Bioplast implant was placed and fixed transversely to the vesicovaginal fascia at the vesicourethral junction (Fig. 1*a*) and was then imbedded by the fascial layers being plicated and brought together in the midline (Figs. 1*b* and 1*c*), using "00" plain catgut. A longitudinal line of fascial infolding catgut stitches was then inserted as far laterally as possible (Fig. 1*d*). Finally, the redundant vaginal wall was cut away and the edges brought together by mattress sutures. A posterior repair was then performed. A Foley catheter was placed into the bladder for three days. If the vagina was atrophic, 1 mg. of oestriol was administered

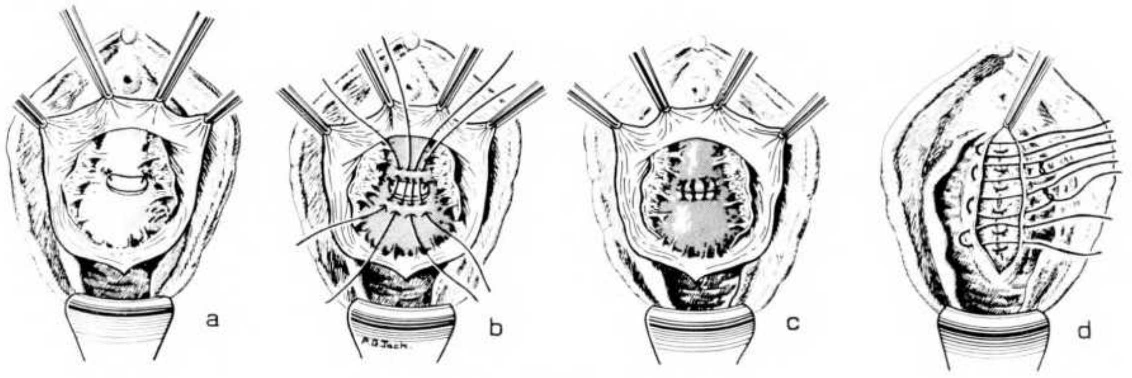

FIG. 1
Operative steps for the insertion of a Bioplast Implant.

daily for a week to promote healing of the vaginal wall. The insertion of Bioplast implant strengthened and elongated the urethra and restored the vesicourethral angle by permanently elevating the bladder neck.

In the years 1968 to 1970, 85 implant operations were performed in the Obstetric and Gynaecological Department of the Semmelweis Medical University, Budapest. Pre- and postoperative gynaecological and laboratory examinations were carried out together with a micturating cystogram. Patients were checked and investigated four times in the first postoperative year and thereafter yearly.

The severity of the stress incontinence was classified as follows: first degree stress incontinence implied leakage with coughing, laughing or sneezing; second degree incontinence meant leakage with milder effort; with third degree stress incontinence there was leakage of urine at rest. Most of the patients were near the end of the reproductive age or did not want a further

TABLE I
*Severity of stress incontinence*

| Parity | Degree of stress incontinence | | |
|---|---|---|---|
| | 1st | 2nd | 3rd |
| Nulliparous | 8 | 1 | 0 |
| Multiparous | 64 | 7 | 5 |
| Mean Age | 47·3 | 51·8 | 57·2 |
| Range | 26–65 | 26–70 | 45–72 |

TABLE II
*Results of operation after an interval of two years related to degree of stress incontinence and previous surgery*

| | | Result of operation accordining to itial degree of stress incontinence | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1st degree | | | 2nd degree | | | 3rd degree | | |
| | | Cured | Failure | Expulsion | Cured | Failure | Expulsion | Cured | Failure | Expulsion |
| No. of previous operations | 0 | 41 | – | 2 | 5 | – | – | 2 | – | – |
| | 1 | 21 | 1 | 1 | 2 | – | 1 | – | 1 | – |
| | 2 | 6 | – | – | – | – | – | 2 | – | – |
| Total (No.) | | 68 | 1 | 3 | 7 | – | 1 | 4 | 1 | – |
| (per cent) | | 94·5 | 1·4 | 4·1 | 87·5 | – | 12·5 | 80·0 | 20·0 | – |

TOTAL CURED: 79 out of 85 (93 per cent)

pregnancy (Table I). Two years after operation 79 out of 85 patients (93 per cent) were cured (Table II). The six unsuccessful operations comprised two implants which failed and four expulsions. The three year follow-up showed similar results.

## ACKNOWLEDGEMENT

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# SUCCESSFUL PREGNANCY AFTER CONSERVATIVE SURGERY AND RADIOTHERAPY FOR DISGERMINOMA OF THE OVARY

BY

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## Summary

A 19-year-old patient was treated for disgerminoma of the right ovary by right salpingo-oophorectomy and pelvic radiotherapy in 1967. The left ovary was shielded after the first two treatments and the patient has since conceived three times and has shown no evidence of recurrence of the tumour.

OVER 85 per cent of disgerminomas occur in patients under the age of 30 years (Herbst, 1974). A disgerminoma is a malignant lesion and reported five-year survival rates vary between 27·1 per cent (Pedowitz *et al.*, 1955) and 86 per cent (Asadourian and Taylor, 1969). Exclusion of tumours containing unusual elements (particularly endodermal sinus tissue, choriocarcinoma or immature teratoma) much improves the overall prognosis. However, unilateral encapsulated tumours have a reported recurrence rate of over 50 per cent after conservative surgery (Pedowitz *et al.*, 1955) and the present report concerns such a case.

### CASE REPORT

A 19-year-old girl presented with a lump palpable in the right groin in November 1966. This was associated with palpable glands in the axilla and neck which, together with a positive Paul-Bunnell test, led to a diagnosis of glandular fever. Though initially the lymphadenopathy resolved, an exacerbation occurred in January 1967 and, one month later, a mass was palpable in the right iliac fossa. The patient was well and her periods were regular. Her full blood count and erythrocyte sedimentation rate were within normal limits and X-rays of the abdomen and chest were normal.

Vaginal examination showed that the mass palpable in the right iliac fossa extended across the pouch of Douglas. At laparotomy in March 1967, a lobulated tumour measuring 17×11×10 cm. and replacing the right ovary was found. The tumour showed no evidence of capsular invasion or fixation but large dilated lymphatics were present in the right infundibulopelvic ligament. A small amount of clear fluid was present in the peritoneal cavity but there was no evidence of spread to other sites in the pelvis, to retroperitoneal lymph nodes or to the liver. The left ovary was bisected, found to be macroscopically normal and reconstituted. The right ovary and Fallopian tube were then removed. The patient made an uneventful recovery.

Pathological examination of the excised specimen showed a tan-coloured mass with tissue broken into lobules by white fibrous strands. Histologically, there was a pure disgerminoma of the ovary (Fig. 1) with tumour infiltrating the ovarian capsule and also a vascular infiltration at the hilum of the ovary.

Post-operative radiotherapy was given in 21 sessions over 30 days from the 28th March 1967,

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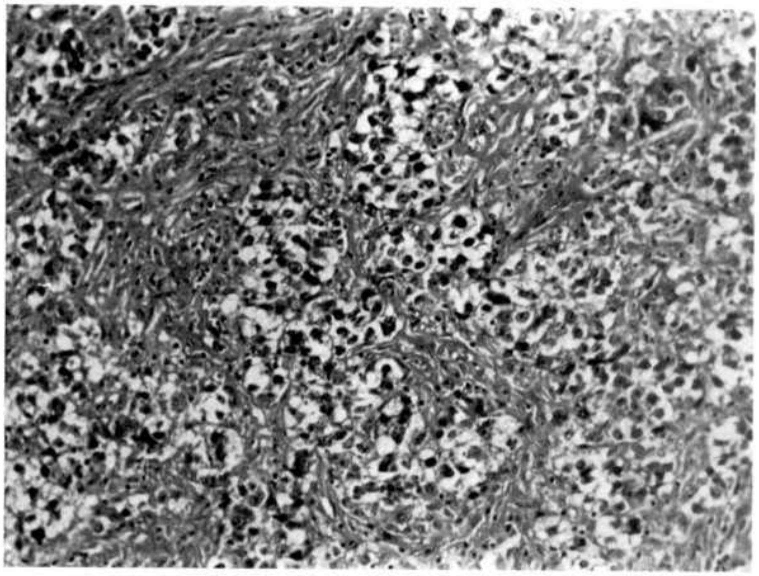

FIG. 1

Disgerminoma cells surrounded by connective tissue septa. (Haematoxylin and eosin ×120.)

on a Mobaltron Cobalt unit. Initially, radiation was directed at the para-aortic and iliac regions covering an area 29 cm. in length, 8 cm. wide at its upper border and 14 sm. wide at its lower border, After two sessions when a dose of only 62 rads had been given, lead was introduced into the beam to shield an area of the left pelvis measuring 13×5 cm. At 1109 rad, additional shielding covering an area 5×2 cm. was used to protect the lower pole of the left kidney. A total midpoint dose of 2034 rads was given to the pelvis, and the remainder of the radiation was directed to the para-aortic region only (over an area of 16×8×8 cm.) to give a total of 3434 rads.

Examination at six-monthly intervals showed no evidence of recurrence and the periods remained regular. The patient got married in April 1971 and, initially, used a contraceptive diaphragm. The first conception occurred in February 1972 but resulted in a spontaneous miscarriage in late April 1972. Her second pregnancy started in December 1972 and followed an entirely uneventful antenatal course. She was admitted to a hospital in spontaneous labour at 38 weeks and had a normal delivery of a healthy normal male child weighing 3·73 kg. The child is currently well. The patient is at present 20 weeks pregnant with no evidence of any complications and fetal growth appears satisfactory.

## DISCUSSION

All agree that if bilateral disgerminoma is present or the tumour is disseminated, the correct treatment is bilateral oophorectomy (with or without hysterectomy) followed by a full course of radiotherapy to the pelvis and para-aortic lymph nodes (Pedowitz *et al.*, 1955; Santesson, 1947; Mueller *et al.*, 1950). It is the treatment of the encapsulated tumour confined to one ovary in the young woman wanting children that causes the most difficulty. Conservative surgery alone for the unilateral encapsulated growth has a recurrence rate of between 43 per cent and 63·2 per cent (Thoeny *et al.*, 1961; Pedowitz *et al.*, 1955) and both groups therefore recommend that all such cases should be treated by radical surgery and radiotherapy. Pedowitz *et al.* (1955) also found that of 64 patients treated conservatively only 18 had a reasonable chance of pregnancy as the remainder needed either excision of the remaining ovary, received radiotherapy or died from dissemination of the tumour soon after the initial surgery. There were only four successful pregnancies following conservative treatment. The opposing view has been put by various authors (Asadourian and Taylor, 1969; Malkasian and Symmonds, 1964). The latter found a five-year survival rate of 80·9 per cent in 27 patients treated by unilateral oophorectomy as opposed to 87·5 per cent in 14 patients treated radically and they believed that this 6·6 per cent drop in survival rate to be acceptable as it allowed 60 per cent of their patients to mature normally or become pregnant. Of the patients who might have conceived, 37·7 per cent subsequently became pregnant. They reported a 52·4 per cent recurrence rate but showed that 63·6 per cent of these responded to further therapy. Asadourian and Taylor (1969) found no difference in the ten-year survival rates between radically and conservatively treated groups.

Another approach is to combine unilateral oophorectomy with conservative radiotherapy. Santesson (1947) at the Radiumhemmet in Stockholm originally used only moderate doses of radiotherapy given over a wide field including the remaining ovary. The addition of radiotherapy to surgery increased the five-year survival rate by 20 per cent and in his series of 15 patients only two became menopausal. Two of his patients became pregnant despite pelvic radiation and other successful pregnancies after large doses of radiotherapy have been reported (Ayerst and Johnson, 1959; Gans *et al.*, 1963). Brody (1961) extended the Stockholm series and included cases of encapsulated tumour in which the remaining ovary was spared while radiotherapy was given to the rest of the pelvis and retroperitoneal fields. Of 40 patients with conservative surgery and radiotherapy, 17 had no direct radiation to the remaining ovary and 10 patients subsequently became pregnant. One of the difficulties in evaluating these combined methods of treatment is that the reported rate of subsequent involvement of the remaining ovary has varied from 8 per cent (Malkasian and Symmonds, 1964) to 36·4 per cent (Pedowitz *et al.*, 1955). Brody (1961) found the incidence of tumour in the remaining ovary to be 7·5 per cent after radiotherapy.

Though Murphy (1929) suggested that radiotherapy before conception could not be shown to affect subsequent children, the Medical Research Council Report (1965) showed that a doubling of the spontaneous rate of mutation occurred between a dose of 15 to 150 rads. Mutations tend to be deleterious (Muller, 1954) but when present in the heterozygons form normally act as a recessive gene and may show no effect until a chance combination with a similar abnormal gene which may be many generations later. Brody (1961) attempted to calculate the increase of genetic abnormalities following conservative radiotherapy and estimated this to be a maximum of ten times the doubling dose to the remaining ovary. He came to the conclusion that it was "reasonably small" —for example he calculated that the risk of "low grade mental deficiency" would rise from 5 per cent to 5·9 per cent, and of autosomal recessive disorders like phenylketonuria, from 0·10 to 0·11 per cent. Eighteen pregnancies occurred in his series, 15 were normal, there was one miscarriage, one abnormal fetus and one termination of pregnancy.

The patient presented here appeared at operation to have a unilateral encapsulated tumour but histological examination showed micro-invasion of the capsule. Thus, the dilatation of the lymphatics in the infundibulopelvic ligament and the histological invasion of the hilar vessels, were taken to indicate a poor prognosis (Pedowitz *et al.*, 1955; Asadourian and Taylor, 1969), and were a major factor in the decision to use postoperative radiotherapy. In retrospect, a wedge biopsy and frozen section should have been examined from the remaining ovary at the time of operation. Asadourian and Taylor (1969) found that four ovaries containing tumour appeared normal on macroscopic examination.

Despite the known sensitivity of the tumour to radiation (Jackson, 1967) it is not suggested that the minimal radiation given to the left ovary in the first two therapeutic sessions was prophylactic. We have no histological or chromosomal studies on the first fetus which aborted spontaneously, but the second child is normal and well; and the third pregnancy seems uncomplicated.

Malkasian and Symmonds (1964) reported that in their patients who become pregnant after treatment recurrence occurred after delivery in one third. However, in most series a five-year survival is regarded as apparent cure since 65 per cent of tumours recur within two years and

95·6 per cent within five years (Asadourian and Taylor, 1969).

## ACKNOWLEDGEMENTS

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# GYNANDROBLASTOMA OF THE OVARY

BY

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## Summary

A gynandroblastoma of the ovary in a 17-year-old girl is reported. Primary amenorrhoea, hirsuties and slight clitoral enlargement were associated with the tumour and high levels of circulating testosterone were found. This is the first gynandroblastoma in which Reinke crystalloids have been identified in the Leydig cell component. The criteria for the diagnosis of this tumour and its histogenesis are discussed.

THE term gynandroblastoma is used to describe a sex cord stromal (gonadal stromal) tumour of the ovary containing readily identifiable cells of the morphological types usually associated with both feminizing and virilizing tumours. Thus, a gynandroblastoma is composed of granulosa cells (nearly always with typical Call-Exner bodies) and Sertoli cell tubules coexisting and intermingling (Serov and Scully, 1973). Most emphasis has hitherto been placed upon the sex cord element of this rare tumour and little has been made of the stromal component. However, a granulosa cell tumour nearly always has theca cells in its stroma, and the usual form of androblastoma is a combination of Sertoli cells and Leydig cells. The "complete" sex cord stromal tumour of mixed cells type should therefore be composed of all four elements: granulosa cells, Sertoli cells, theca cells, and Leydig cells, but such a tumour has not yet been described (Teilum, 1971). The gynandroblastoma reported here was a unique combination of granulosa cells, Sertoli cells and Leydig cells, the last containing crystalloids of Reinke. (These are eosinophilic, crystalline, rod-like structures in the cytoplasm of the cells. They are not sudanophilic and not refractile and are thought to be protein bodies, perhaps developing from pre-existing spherical cytoplasmic inclusions. Reinke crystalloids are seen in only a small proportion of Leydig cells of the ovary and testis, but are not found in any other cell type, so their identification is of diagnostic importance.) As well as these unequivocal cell types, the stroma contained cells which were probably of thecal type, making this the most complete example of a gynandroblastoma yet described.

### CASE REPORT

The patient was first seen when she was 17 years old because she had never menstruated. Breast development had started at the age of 11 and pubic and axillary hair growth had occurred at the same time. Her medical history contained

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TABLE I

*Plasma and urinary hormone levels*

| | Plasma Testosterone (ng. per 100 ml.) | Plasma Cortisol (μg. per 100 ml.) | Urinary 17-oxosteroids (mg. per 24 hours) | Urinary 17-hydroxy-corticosteroids (mg. per 24 hours) |
|---|---|---|---|---|
| Preoperative | 292 | 8 | 9 | 10 |
| After dexamethasone | 216 | 5 | – | – |
| Postoperative | 40 | 7 | 3 | 5 |
| Normal range in females | 15–70 | 6–22 | 4–10 | 4–17 |

nothing relevant and there was no family history of amenorrhoea or late menarche. She was of average height and normal build, with slight facial hirsuties and a pubic hair line of male distribution. The breasts and nipples were well developed. No abnormality was detected on vaginal examination but the clitoris was slightly enlarged. At laparoscopy an ovarian tumour (about 5 cm. in diameter) was seen on the left. The pelvis was otherwise normal. No endometrium was obtained on curettage.

To avoid interfering with the patient's studies, laparotomy was delayed for three months, during which time she had three rather prolonged but light periods. When laparotomy was carried out, the presence of a left ovarian tumour was confirmed and the whole ovary was removed. The right ovary had a normal appearance and a wedge biopsy was taken from it. Curettage was also performed and this time some endometrial tissue was obtained. The postoperative course was uneventful and she subsequently had regular periods of normal duration, accompanied, unlike the few previous ones, by slight dysmenorrhoea.

*Biochemical findings* (*Table I*)

There was a very high plasma testosterone level which fell to normal within 24 hours of removal of the tumour. At the time of operation, blood was taken from the left ovarian vein and the testerone concentration in this sample was 5,160 ng./100 ml.

*Pathology*

The ovary was replaced by an oval mass (6 cm. in greatest diameter) to which some Fallopian tube was attached. The smooth capsule did not appear to be penetrated by tumour which, on sectioning, was solid and lobulated, the lobules being pink with a yellowish hue and separated by fibrous septa. No normal ovarian tissue was apparent.

The microscopic pattern of the tumour varied from place to place. The well differentiated areas had two main patterns, the first of which consisted of solid groups of small, dark, polyhedral cells. In most of these cell masses there were several round spaces of various sizes (Fig. 1) characterizing them as the microfolliculoid pattern of granulosa cell tumour with

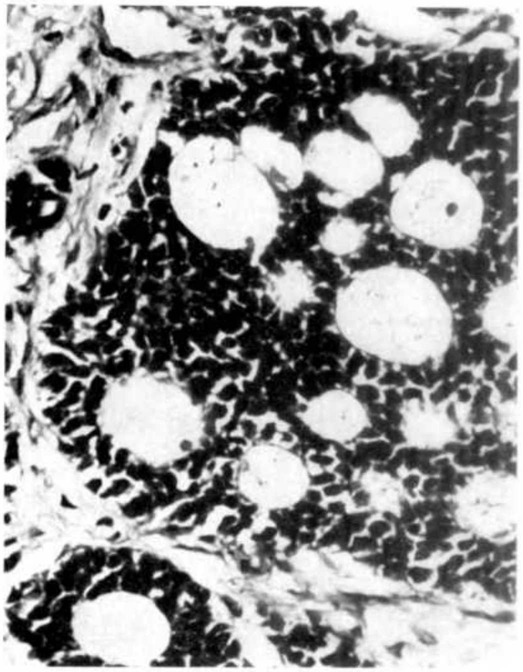

FIG. 1

Granulosa cell aggregates with typical Call-Exner bodies. (Haematoxylin and eosin × 320.)

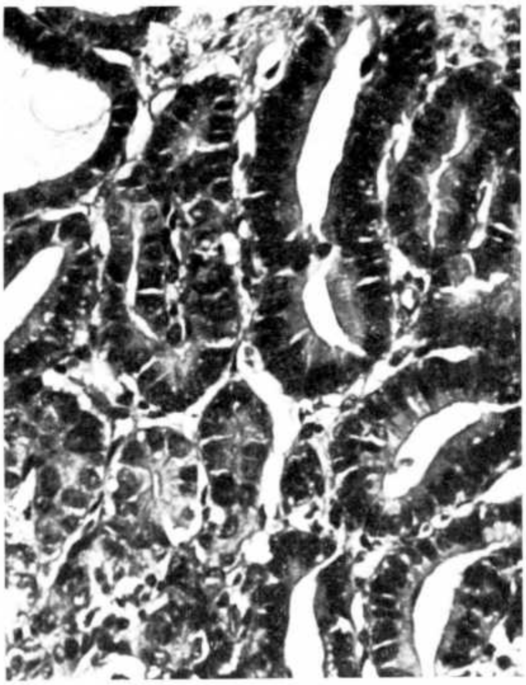

FIG. 2
Tubules lined by tall, columnar Sertoli cells.
(Haematoxylin and eosin ×320.)

typical Call-Exner bodies. The second pattern comprised tubules lined by a single layer of fairly tall columnar cells (Fig. 2), identical in appearance to the Sertoli cell tubules of a Sertoli-Leydig cell tumour. Throughout the tumour these two patterns and cell types were found together (Fig. 3). The stroma consisted mainly of fibrous tissue, although in some areas the stromal cells were rather plumper and of thecal type (Fig. 4). The most striking combinations of cell types were seen in the areas which were slightly less well differentiated. Here the Sertoli cell tubules and granulosa cell cords were intimately mixed and there were some tubules which were lined partly by a single layer of Sertoli cells and partly by groups of granulosa cells (Fig. 5). The stroma contained, in addition to thecal cells, fairly large round cells with round or oval nuclei and slightly granular, eosinophilic cytoplasm. The demonstration of crystalloids of Reinke (Fig. 6) confirmed that these were Leydig cells.

A fat stain (oil red O) demonstrated abundant neutral lipid in the cytoplasm of the Leydig cells. Fat was also present, although in smaller amounts, in some of the plumper, elongated stromal cells and in the Sertoli cells. None was seen in the granulosa cells.

The endometrium was in the proliferative phase and showed no abnormalities. The biopsy of the right ovary showed normal ovarian cortex with many primordial follicles and atretic follicles. No tumour was present.

## DISCUSSION

Since Meyer (1930) first described the gynandroblastoma there have been occasional reports of tumours of this type (Plate, 1938; Mechler and Black, 1943; Hobbs, 1949; Emig *et al.*, 1959; Ross *et al.*, 1959; McBride *et al.*, 1962; Neubecker and Breen, 1962; Hartford and Russell, 1963; Ferrand *et al.*, 1965; Novak, 1967; Pearson and Jaffurs, 1970). Fox and Langley (personal communication) accept only eight tumours as authentic gynandroblastomata. Many of the earlier cases are unacceptable because they

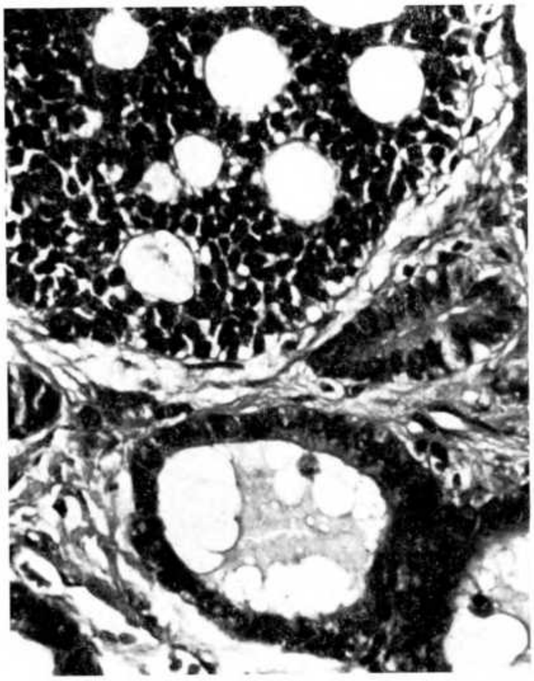

FIG. 3
Sertoli cell tubules and granulosa cells in close proximity. The stroma in Figures 1, 2 and 3 is non-specialized fibrous tissue.
(Haematoxylin and eosin ×320.)

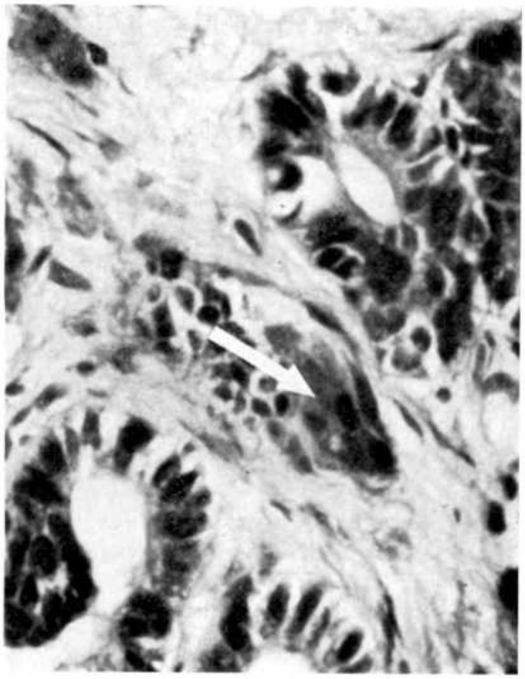

FIG. 4

Stroma containing plump cells (arrow) with eosinophilic cytoplasm. These are probably theca cells. (Haematoxylin and eosin ×450.)

do not satisfy the histological criteria which are now considered necessary for diagnosis, the emphasis often having been on the hormonal activity of the tumours. Ross *et al.* (1959) described a tumour causing postmenopausal bleeding and associated with hirsuties. They called it a gynandroblastoma because of its apparently ambiguous hormonal effect, but their illustrations suggest that it was a granulosa cell tumour. Similarly, Hughesdon and Fraser (1953), in a review of 38 supposed gynandroblastomata, found three that were pure granulosa cell tumours with a masculinizing effect. Mechler and Black (1943), reporting an authentic gynandroblastoma, considered that the term was useful to describe a clinico-pathological syndrome, but that there was no accompanying histological pattern. However, production of both oestrogen and androgen by a tumour is not diagnostic of gynandroblastoma, as testicular elements may be feminizing and ovarian elements may be masculinizing (Govan, 1973). For this reason, Morris and Scully (1958) took the opposite view to Mechler and Black (1943) and maintained that the diagnosis should be determined by the morphology of the constituent cells, irrespective of the hormonal activity of the tumour. Their suggestion has been adopted by subsequent authors (Scully, 1968; Teilum, 1971; Serov and Scully, 1973).

For the diagnosis of gynandroblastoma to be made, it is necessary to find readily identifiable granulosa-theca cell elements and androblastomatous elements and, so that these cell types can be readily distinguished, the tumour must be well differentiated. In addition, all the constituent cell types must be intimately mixed, as separate Leydig cell and granulosa cell tumours have been found in the same ovary (Dougherty *et al.*, 1958). The tumour which we describe here satisfies these rather rigid diagnostic requirements and is the first gynandroblastoma in which undoubted Leydig cells with Reinke crystalloids have been found together with the more usual Sertoli cell tubules and granulosa

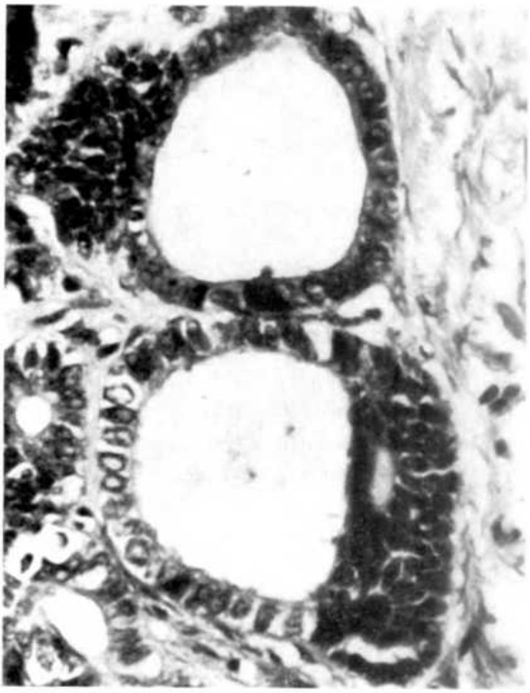

FIG. 5

Two tubules lined partly by Sertoli cells and partly by granulosa cells. (Haematoxylin and eosin ×385.)

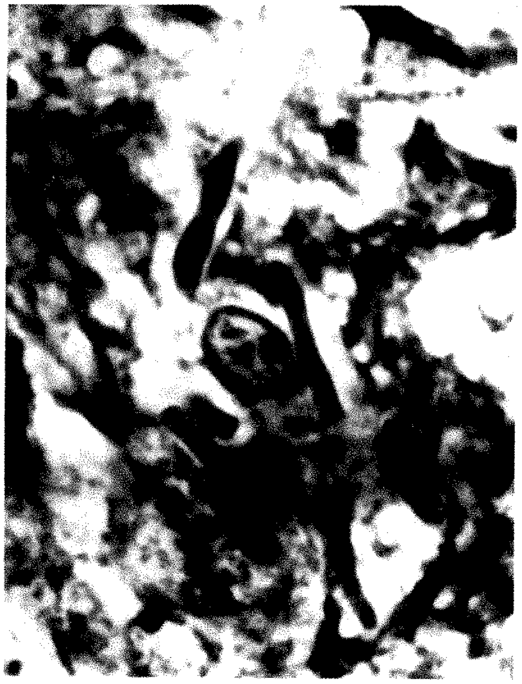

FIG. 6

Reinke crystalloids around the nucleus of a Leydig cell in the stroma.
(Heidenhain's iron haematoxylin × 1440.)

cell islands. The constituent elements were well mixed and testicular and ovarian cell types were present in roughly equal proportions. The dominant functional cell, however, was probably the Leydig cell, producing high levels of testosterone. Although it must be accepted that oestrogens or androgens may be produced by any of the tissues of the ovary in suitable circumstances, there is evidence to suggest that the Leydig (hilus or interstitial) cell is mainly responsible for androgen production (Govan, 1973). It is interesting that in this patient the high levels of testosterone had only a minimal masculinizing effect as well as inhibiting menstruation.

The origins of the gynandroblastoma are not clear. Plate (1938) thought that it was a tumour of indifferent germinal epithelium whereas Mechler and Black (1943) suggested a teratomatous origin. From the embryology of the gonads, it can be argued that Sertoli and Leydig cells can be derived only from the medulla of the primitive gonad and that granulosa cells develop only from the sex cords of the cortex (Langley, 1973). If this theory is accepted, then a gynandroblastoma can only develop if medulla and cortex are both stimulated at the same time to develop their latent capacities. For this reason, Scully (1953) felt that a true intermingling of male and female cell types was unlikely. Most recent authors, however, assume that the gynandroblastoma is derived from the indifferent gonadal mesenchyme which possesses the ability to manifest bisexual potentialities and so differentiate into both male and female structures (Emig *et al.*, 1959; McBride *et al.*, 1962; Neubecker and Breen, 1962; Teilum, 1971), a suggestion which has also been put forward to explain the origin of pure Leydig cell tumours (Merrill, 1959; Anderson, 1972). Finding apparent female and male cells so closely mixed together and even lining the same tubules (Fig. 5) seems to us to support the suggestion of divergent differentiation from a common precursor.

As so few tumours of this type have been seen and followed up, it is difficult to assess the prognosis. Novak (1967) records one death from gynandroblastoma, but other patients are alive and well for periods of up to ten years with partial or complete relief of their endocrine symptoms. There seems no reason why the behaviour of a mixed sex cord stromal tumour should be any different from that of its constituent elements and, as a gynandroblastoma must be well differentiated, the malignant potential is probably low.

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# CYTOLOGICAL DIAGNOSIS OF A PRIMARY MALIGNANT MELANOMA OF THE VAGINA

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## Summary

The cytological appearances of a primary malignant melanoma of the vagina are described. A diagnosis of melanocarcinoma, made from smears showing malignant cells with melanin pigment in their cytoplasm, was confirmed histologically.

PRIMARY malignant neoplasms of the vagina are rare, comprising about 1 per cent of all primary genital malignancies (Novak, 1966), and only a small minority are primary malignant melanomas. Ehrmann *et al.* (1962) could only find 34 reported cases, while the number diagnosed cytologically are much smaller (Linthicum, 1971; Yamada *et al.*, 1972).

### CASE REPORT

A 44-year-old nulliparous maiden lady presented with frequent periods, intermenstrual bleeding for three months and continuous bleeding for the last 27 days. Her mother had died from endometrial carcinoma.

Vaginal examination was difficult because of the intact hymen. The cervix looked healthy, but a necrotic lesion was noticed on the left lateral vaginal wall. Smears were made and she was admitted for removal of the nodule and a curettage.

Under general anaesthesia, a black papillomatous mass measuring 2×2 cm. was seen at the junction of the middle and lower thirds of the vagina. Since the tumour appeared undisseminated a wide local excision was performed (Olsen, 1966). A curettage of the endometrial cavity was also made. Both cytological and histological reports confirmed the diagnosis of malignant melanoma of the vagina. The endometrium was normal. There was no evidence of secondary spread and no enlarged inguinal lymph nodes were felt. The liver was not palpable. There was no evidence of a primary growth elsewhere. A chest X-ray was negative.

A hysterovaginectomy with en block dissection of the inguinal nodes was considered, but it was decided merely to keep the patient under careful surveillance because there appeared to be no secondary invasion, and because spontaneous regression can occur (Everson and Cole, 1966), an outcome attributed to the immunological effect stimulated by some melanomas (Bodenham and Hiles, 1971).

Fifteen months later the patient was well and the pelvis was clear. The vagina was healthy with no evidence of recurrent tumour. Vaginal wall smears taken from the operation site were all negative.

### *Cytology*

The cells exfoliated from the malignant melanoma resembled those in the tissue sections. The cells were mostly oval or round and varied greatly in size (Fig. 1). In some cells the nuclear/cytoplasmic ratio was not greatly altered, and

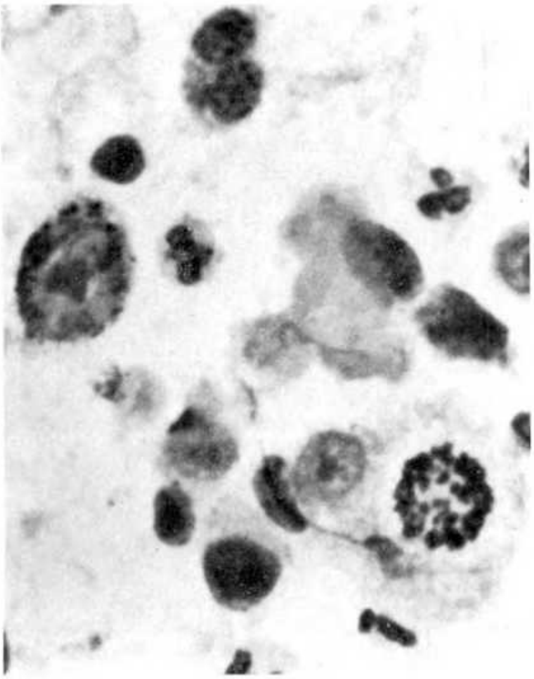

FIG. 1

Cells exfoliated from the malignant melanoma showing variation in size and shape. Mitosis can be seen in one cell. (Papanicolaou Stain × 500.)

in approximately 40 per cent of the cells a brown, finely granular, sometimes coarse pigment was seen in the cytoplasm (Fig. 2). The nuclei exhibited malignant characteristics especially huge nucleoli. Some mitotic activity was observed (Fig. 1). Contrary to the report by Graham (1963) that malignant melanoma of the vagina does not desquamate cells, we found large numbers on the smears and noted that there seemed to be an almost total lack of cohesion of cells (Hajdu and Savino, 1973). Although this cell morphology appears characteristic of malignant melanoma, special staining techniques such as Masson Fontana or Schmorl's Reaction for positive identification of the melanin pigment should be used (Fig. 3).

## DISCUSSION

Melanocarcinoma is the malignant neoplasm of the melanin producing cells—the melanocytes. It is thought that they migrate to the epidermis from the neural crest. Melanin synthesis begins in these cells with the tyrosinase-catalyzed hydroxylation of tyrosine to dihydroxyphenylaline (DOPA), which is coupled with protein to produce melanin (Wellings and Siegel, 1963).

Approximately 85 per cent of malignant

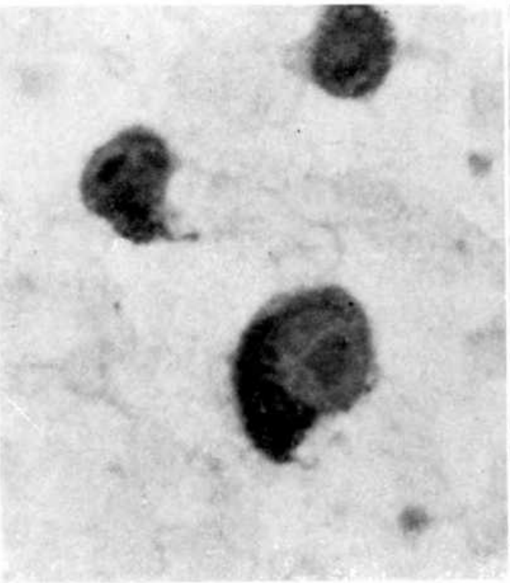

FIG. 2

Malignant cells containing melanin pigment in their cytoplasm. (Papanicolaou stain × 500.)

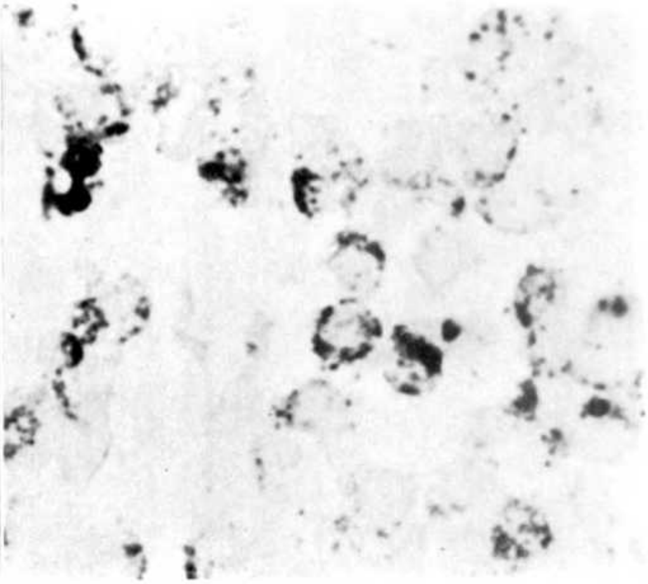

FIG. 3

Melanin pigment specifically demonstrated. (Masson Fontana × 500.)

melanomas arise in the skin, the most common extracutaneous sites of origin being the eye, oronasopharynx, larynx, bronchus, oesophagus, gall bladder, genitalia and anorectal region (Anderson, 1971). Metastasis usually occurs at first through the lymphatic system.

Surgery is still the major method of treatment of malignant melanomas, but chemotherapy, radiotherapy and endolymphatic therapy may also be utilized. Immunotherapy is a possibility for the future and reasonable success has already been reported in the treatment of human melanomas with BCG (Gutterman *et al.*, 1973).

However, whatever form of treatment is used, it must always be remembered that malignant melanoma is notorious for its unpredictable behaviour, and while spontaneous tumour regression occurs in a small number of patients (Everson and Cole, 1966) the outcome for most is fatal (Goldsmith *et al.*, 1972).

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*British Journal of Obstetrics and Gynaecology*
Jan. 1975. Vol. 82. pp.77–79.

# CONCURRENT OVARIAN AND NORMAL INTRAUTERINE PREGNANCY

BY

ABDULATIF YASSIN KASSAB
*Consultant in Obstetrics and Gynaecology*
*Military Maternity Hospital, Damascus, Syria*

## Summary

A patient with concurrent ovarian and intrauterine pregnancy is described. Certain features were unique.

HERTIG (1951) suggested that the incidence of combined extrauterine and intrauterine pregnancy was 1 in 25 000 to 1 in 40 000 pregnancies. Eyton-Jones (1968) traced six concurrent ovarian and intrauterine pregnancies dating back to 1940 and recorded a seventh case. The present report would appear to be the furthest advanced ovarian pregnancy and the largest fetus delivered after an apparently normal home confinement.

## CASE HISTORY

A multiparous woman, aged 32, was seen because of abdominal pain. Her obstetric history was: a twin abortion at 12 weeks; a premature labour at 32 weeks; and four normal pregnancies.

At 32 weeks gestation in the last pregnancy the patient had a severe pain in her right iliac fossa and lower abdomen and fainted. A diagnosis of urinary tract infection was made by her local doctor and she was treated with penicillin but she continued to feel generally unwell. Her appetite was poor and she lost 10 kg. in weight in the last eight weeks of pregnancy. At full term she was delivered uneventfully at home, under the care of the local midwife, of a healthy female infant weighing 3 kg. One month after delivery she presented complaining of abdominal swelling associated with pain in the right iliac fossa during defaecation. There were also episodes of constipation alternating with diarrhoea. These symptoms had apparently dated from the 32nd week of the last pregnancy. She had not menstruated since the confinement.

The patient was thin and pale, and her general condition was poor. Her haemoglobin was 10 g./100 ml. Prior to the last pregnancy she had weighed 60 kg., but her body weight was now 50 kg. An irregular mass extended from the pelvis to three fingers above the umbilicus and towards the right iliac fossa. It had a smooth but lobulated surface and was not tender. No free fluid was demonstrated in the peritoneal cavity. Vaginally, the uterus was anteverted but bulky, and the left appendage normal. The presence of the mass was confirmed and it was slightly tender.

An X-ray of the abdomen showed the presence of fetal parts (maturity 22 weeks) in the right iliac fossa. A hysterosalpingogram (Fig. 1) showed the uterus to be of normal shape and pushed to the left by the mass containing the fetus. The right tube was elongated and stretched over this mass. Both tubes were patent.

At laparotomy the mass was found to be replacing the right ovary. It was attached to the uterus by the utero-ovarian ligament and to the right pelvic wall by the infundibulo-pelvic ligament. The right tube was elongated and stretched over the mass but appeared normal. Filmy adhesions surrounded the mass. The uterus was bulky and anteverted and the right appendage was normal. Exploration of the abdomen revealed no other abnormality and the mass was removed *in toto*. There were some adhesions between the mass and the rectum which required division by sharp dissection and the rectal wall

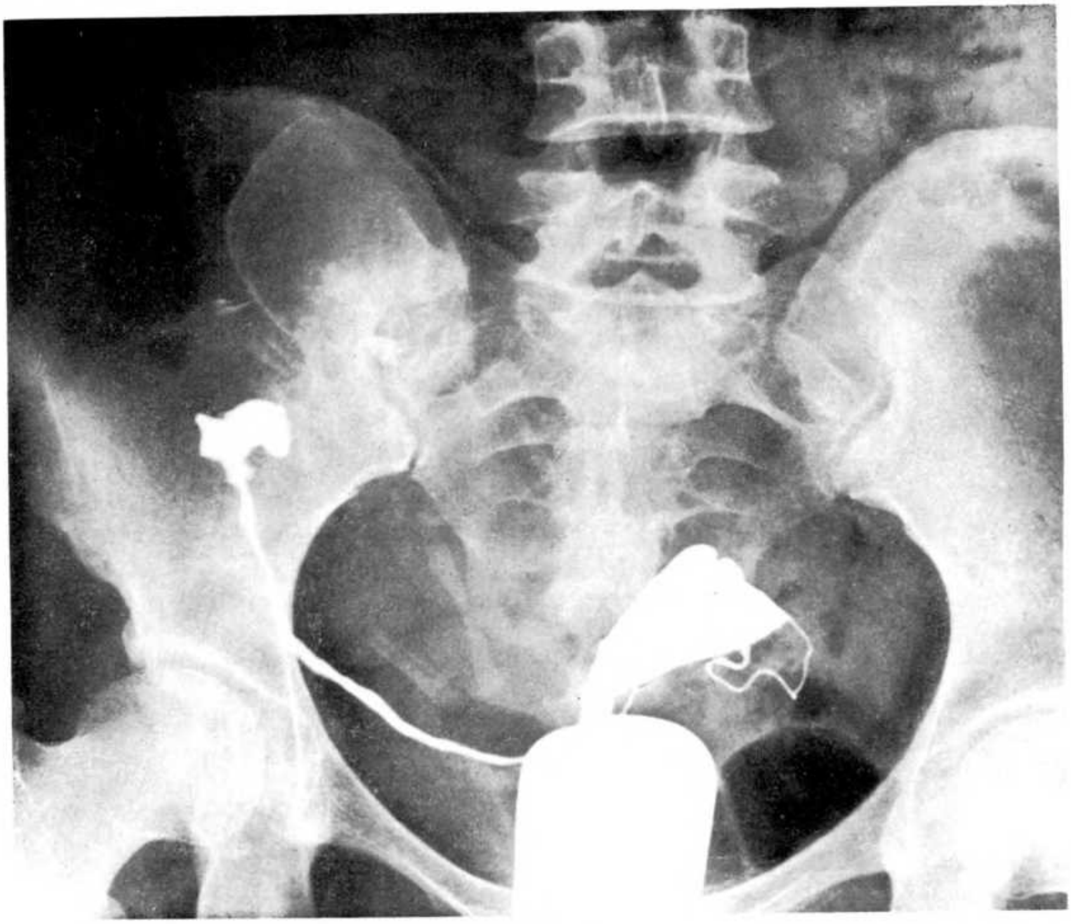

FIG. 1

Hysterosalpingogram performed one month after normal delivery.

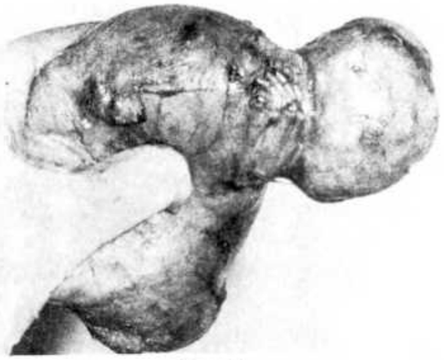

FIG. 2

The mass after removal. Fetal parts can be seen through the covering membrane.

was repaired with three interrupted catgut sutures. The abdomen was closed in layers. No blood transfusion was required.

The postoperative period was uneventful and six weeks later the patient's general condition was very satisfactory. She has since had an uneventful normal pregnancy.

*Specimen*

The oval mass, 6 × 7 × 15 cm. was covered by a membrane and had the shape of a fetus. It weighed 250 g. The skull bones could be seen through the membrane which when incised revealed the fetus (Fig. 2). This was macerated and both the cranial and thoracic cavities were collapsed. There was a lower dorsal kyphosis with hemivertebrae and a left talipes equinovarus (Fig. 3).

Microscopically, sections of the outer coverings showed numerous chorionic villi rimmed by a strip of ovarian stroma. No germinal epithelium or ovarian follicles were seen.

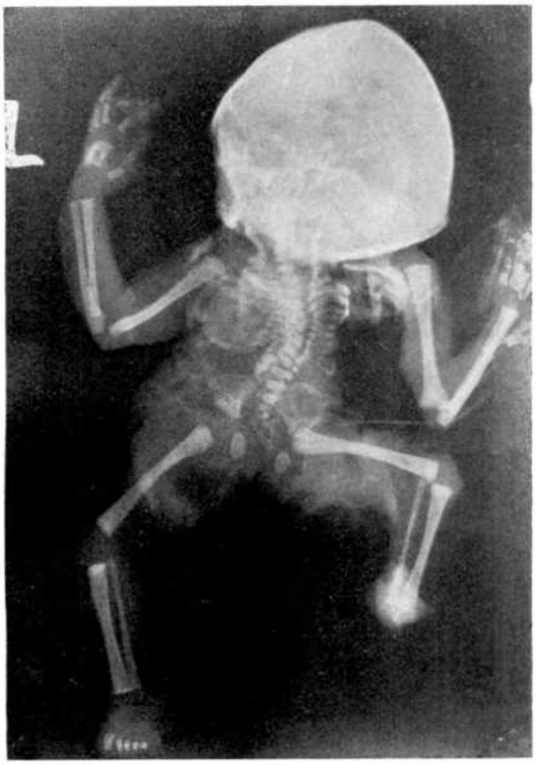

FIG. 3

Radiological examination of the specimen removed at laparotomy.

## COMMENT

This patient satisfied the four criteria of Spiegelberg (1878) for ovarian pregnancy which were that the tube should be intact, that the gestation sac should occupy the normal position of the ovary and be connected to the uterus by the utero-ovarian ligament and that ovarian tissue be demonstrable in the wall of the sac. The condition made by Rubin (1951) that histological confirmation should be obtained was also fulfilled. Presumably the ovarian pregnancy died at the time of the abdominal symptoms when the intrauterine pregnancy was 32 weeks by date.

## ACKNOWLEDGEMENTS

I thank Professor J. C. McClure Browne, Mr. W. G. MacGregor, and Miss E. M. Mills for their advice and encouragement in the preparation of this case.

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# BOOK REVIEWS

**The Morality of Killing. Sanctity of Life, Abortion and Euthanasia.** By MARVIN KOHL. Pp. xiv+112, with figs. and tables. Peter Owen Ltd., London, 1974. Price: £3.25.

IN the preface to this book the author states that his purpose in writing it was, first, to provide a more adequate formulation of the principles of the sanctity of life; second, to explain the fundamental issues and thereby help to resolve the abortion controversy: and, third, to examine the notion of kindly killing and make the case for beneficent euthanasia. He then says that the book is in fact a series of chapters that were written separately and stand as independent units. It is doubtless this latter fact that leaves the reader with a lack of continuity and cohesion. If the prospective reader is interested in a series of highly technical abstruse philosophical discourses on such diverse subjects as the sanctity of life, moral killing, kindness and women's liberation he will find this volume much to his liking. If, on the other hand, he is interested in the practical medical and legal problems involved in abortion and euthanasia he will find nothing whatever to help him resolve his difficulties. Beneficent euthanasia is all very well as a basic philosophical principle, but philosophical principles are not necessarily the best basis for formulating legislation or for deciding who should make decisions. The author dismisses too lightly the effects on society, as well as on the individual, of legislation, and perpetuates the idea that death is always suffering and agony. Similarly, the philosophical argument about whether or not a fetus is a human being cannot resolve the abortion issue whatever belief you may hold. This is a book for the student of philosophy rather than for the layman or the practising doctor. The author's final conclusion—that all human beings at one time or another need the assistance of others and that when it is both necessary and reasonable to help it is our obligation to do so—cannot be faulted, but it evades the issue of who decides what form that help should take. The philosophical arguments on both these topics have been going on since Plato first enumerated them 2000 years ago and, fascinating as they are, they offer little to the resolution of the difficulties surrounding both these emotive topics.

*Sir John Peel.*

**Eugenic Sterilization.** Edited by JONAS ROBITSCHER. Pp. xi+146, with figs. and tables. Charles C. Thomas, Springfield, Illinois, U.S.A., 1973. Price: $7.95.

DISCUSSED in this book are current American attitudes towards eugenic sterilization. It deals with the political and social implications of compulsory sterilization, psychiatric, moral and religious points of view and the legality of the procedure. The legal aspects apply in the main to American jurisprudence. The chapter on genetics although short, highlights the poorly founded genetic basis for eugenic sterilization and the author comments that "conditions such as feeble mindedness lumped together have no genetic components and apart from the complexities of dealing with elimination of genes from the population, error is compounded by the unfounded criteria of measurement of mental capacity". People concerned with eugenic sterilization will find this book a valuable source of reference and those contemplating an individual case would do well to read it so as to see this problem in its wider perspective. The monograph succeeds in presenting a well written and comprehensive survey of the subject.

*B. A. Lieberman.*

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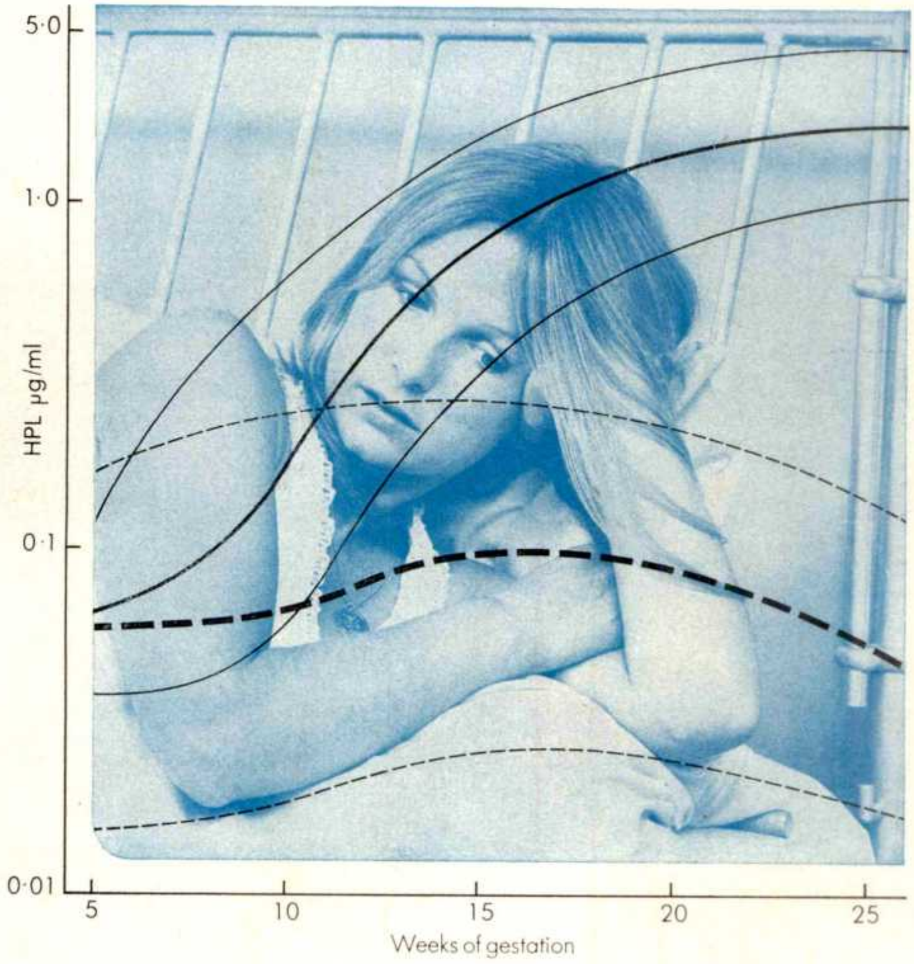

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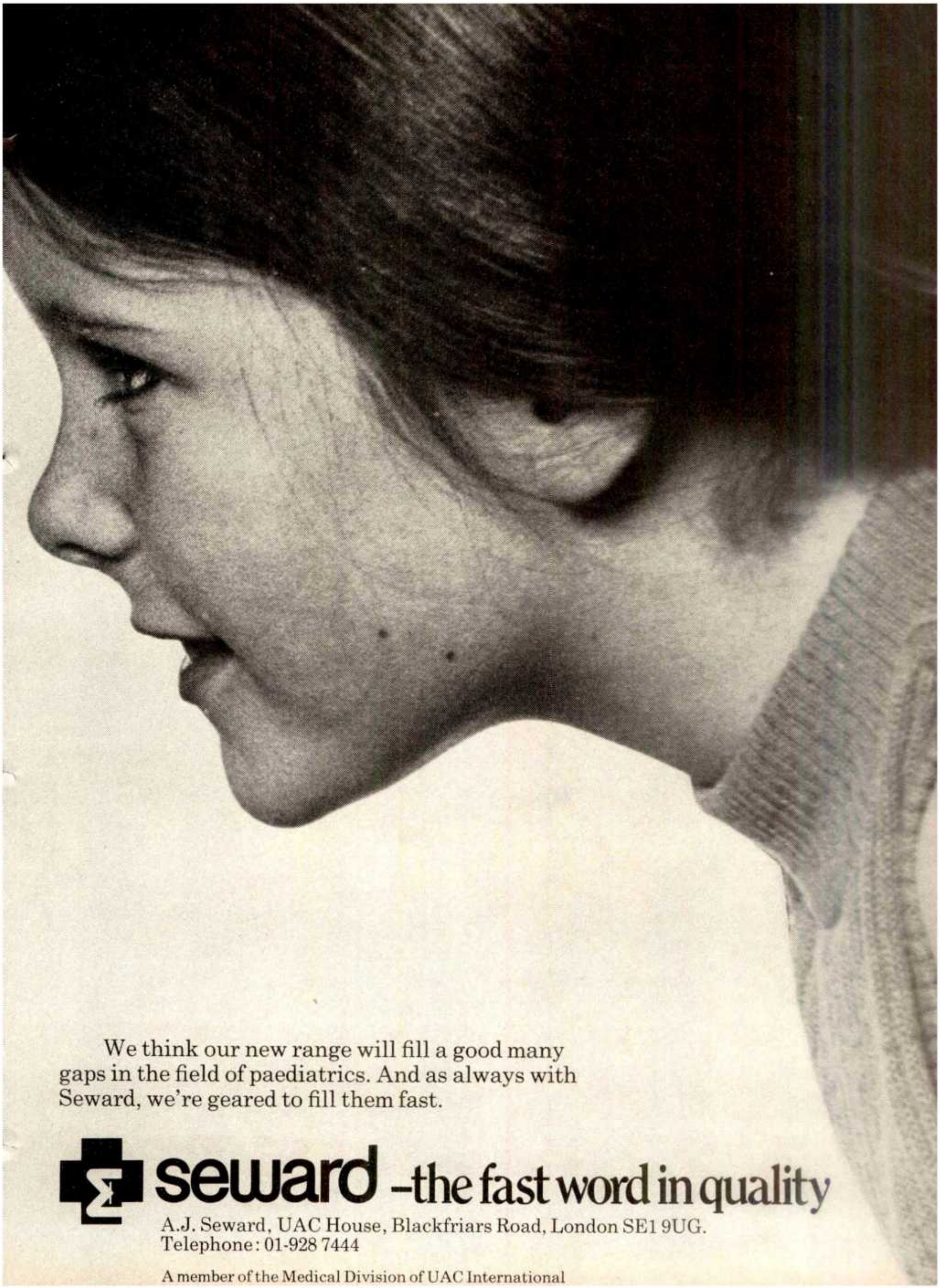

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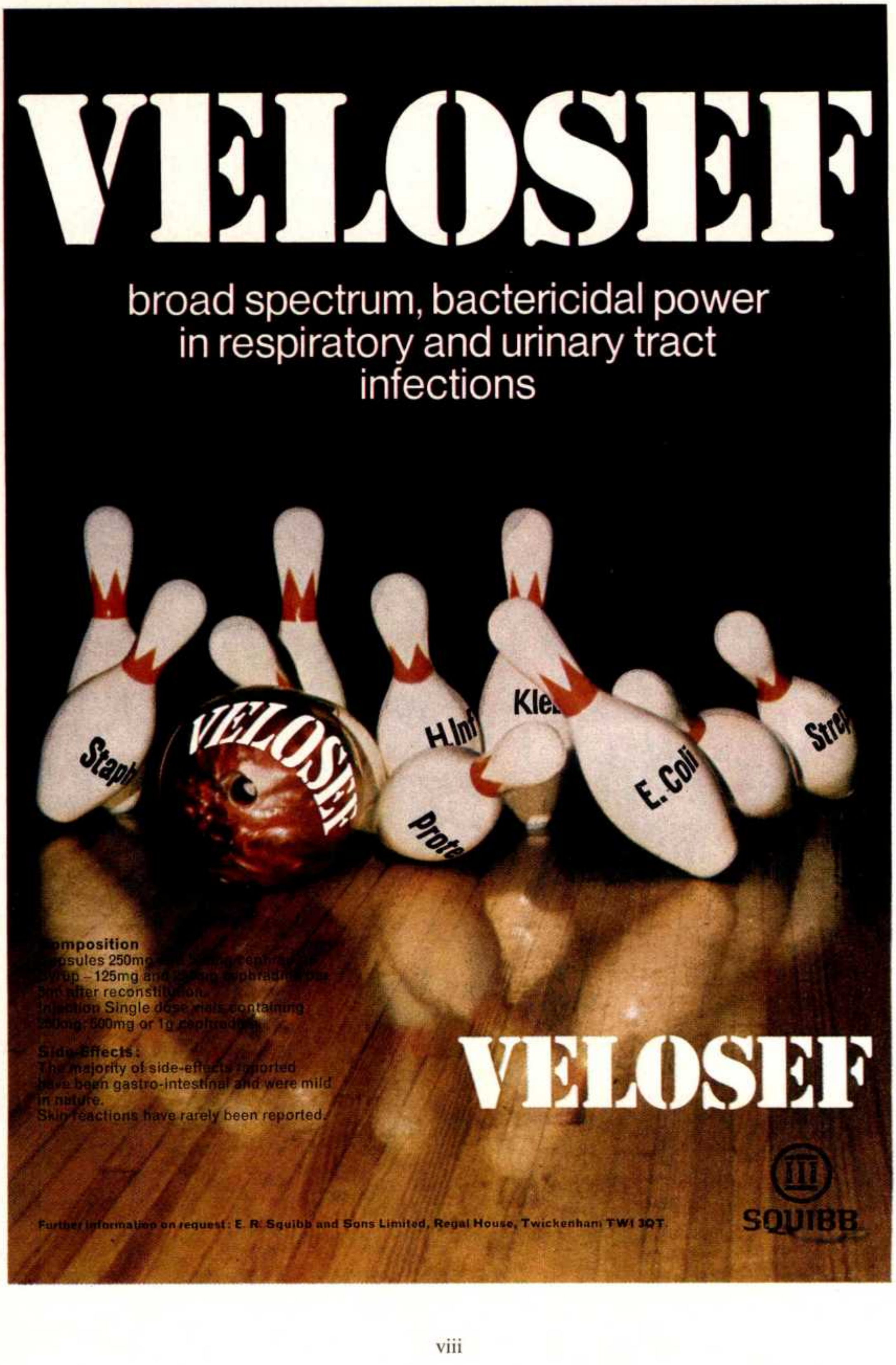

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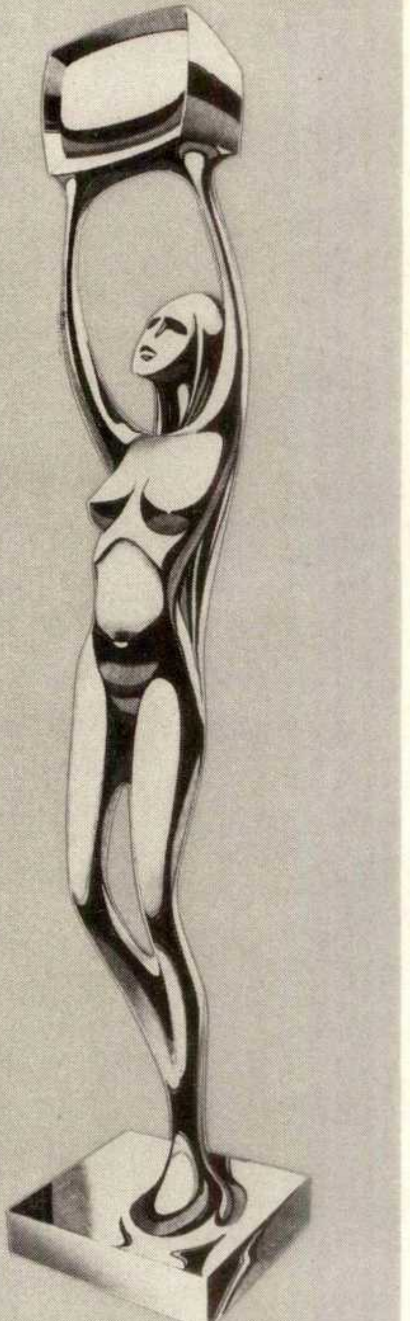

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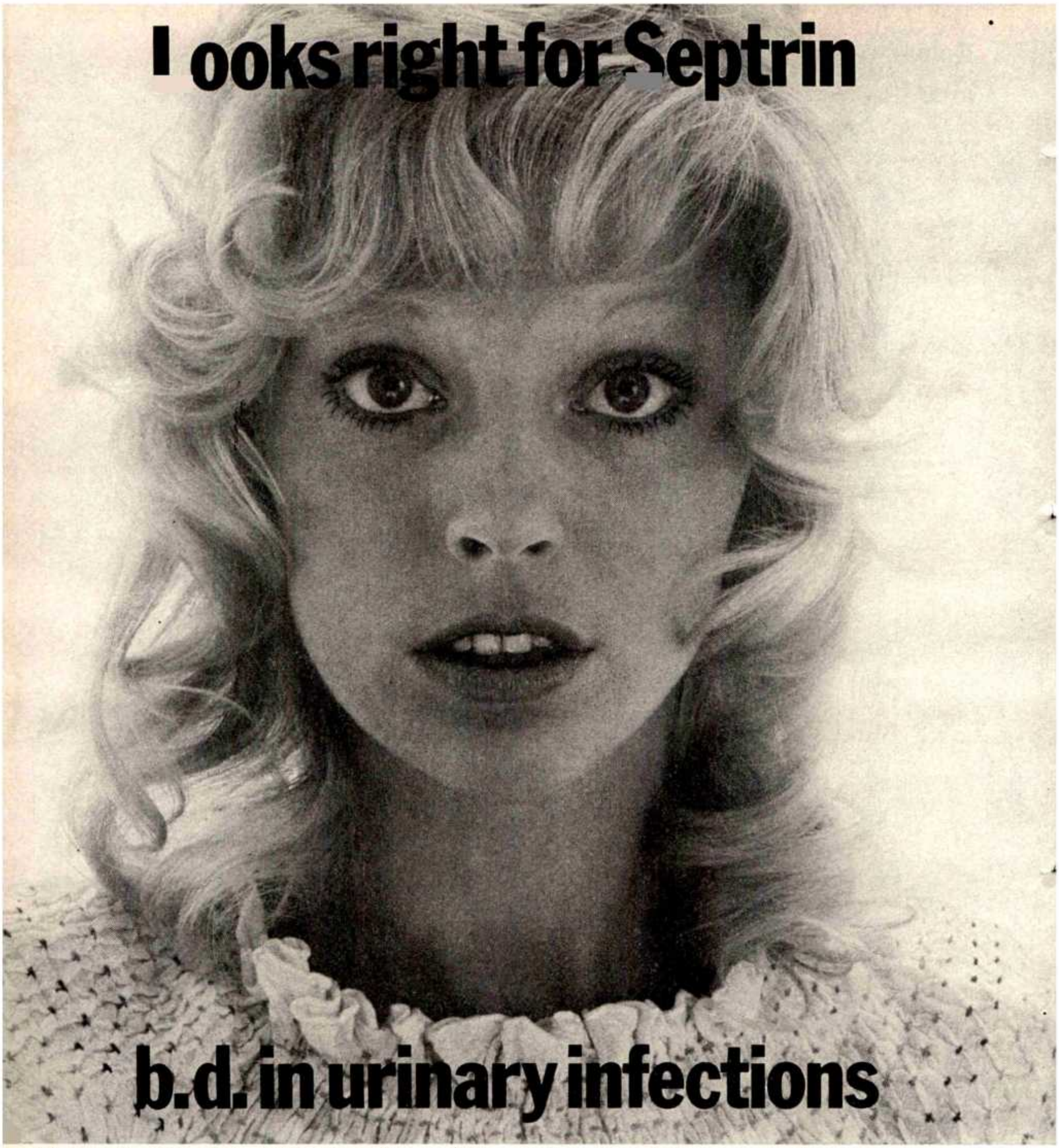

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3 *Clin Trials J*, (1972) **9/3**, 9.

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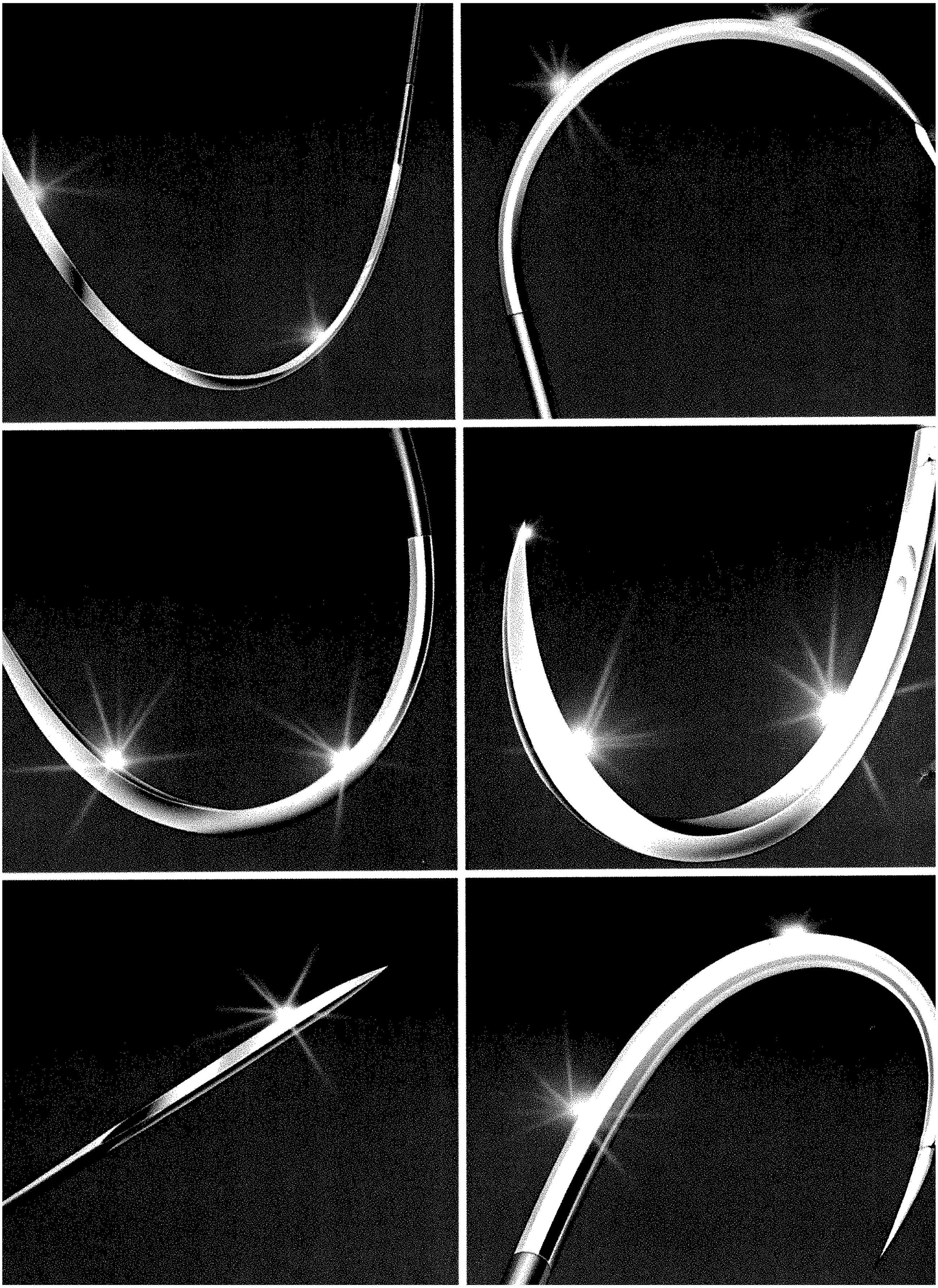

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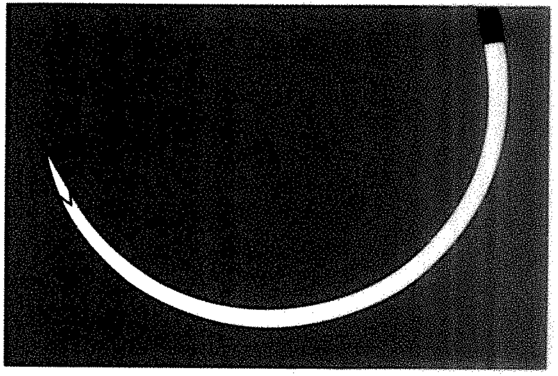

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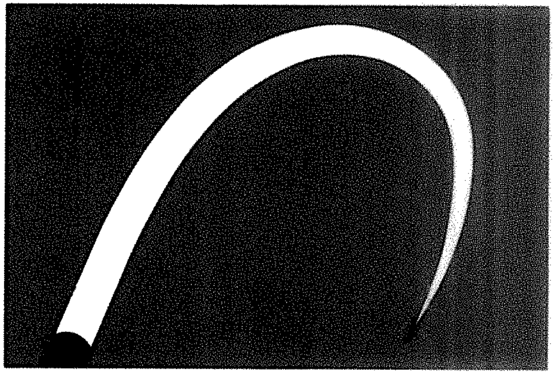

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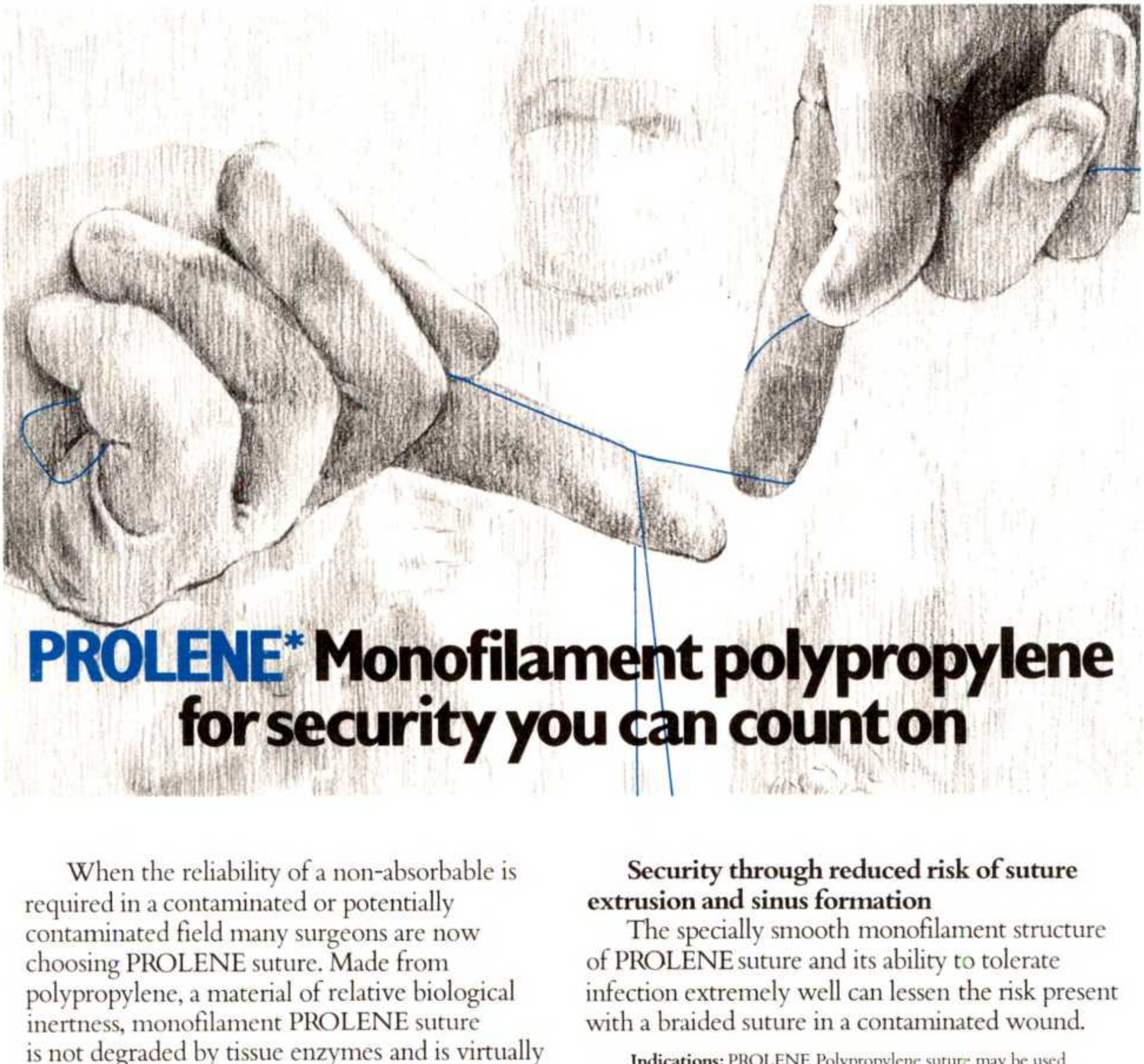
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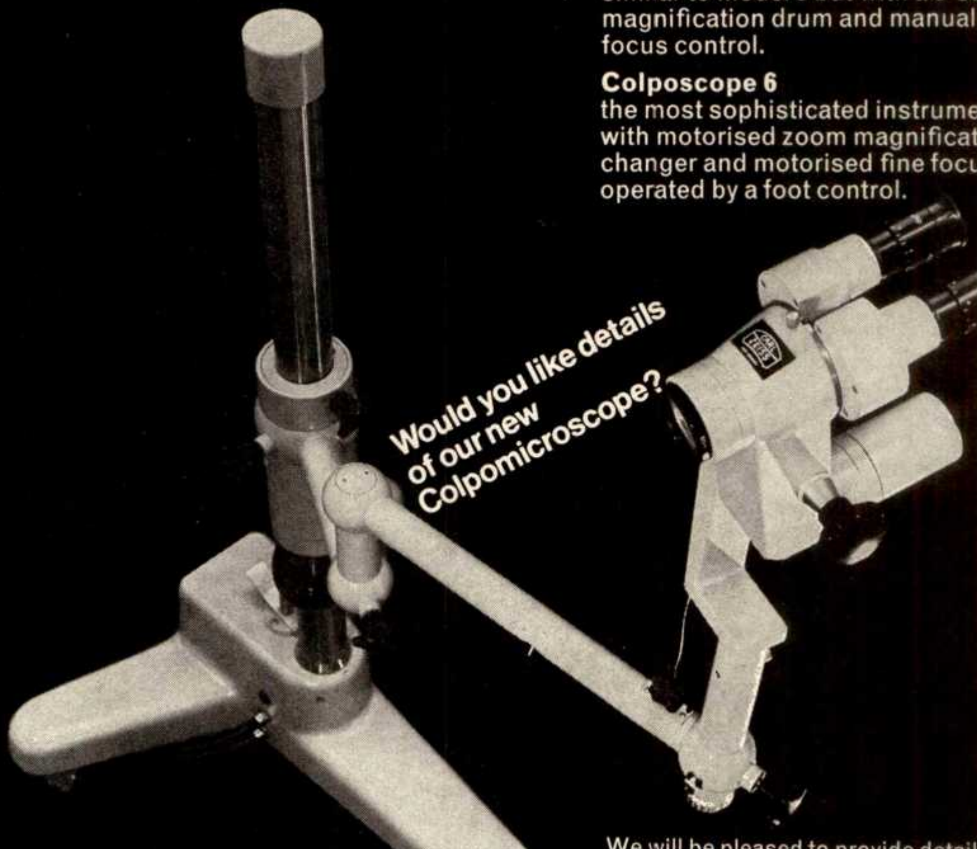

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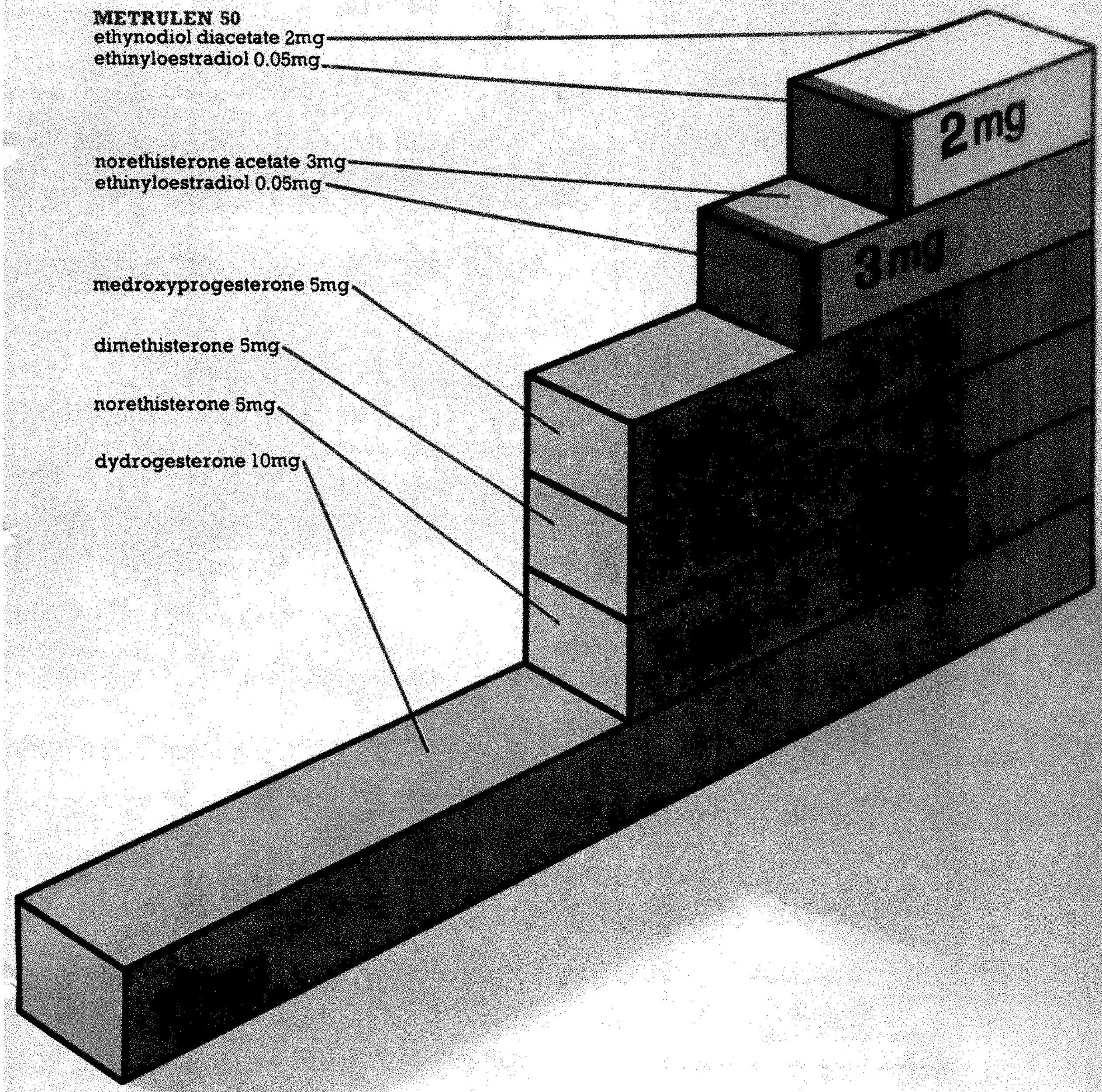
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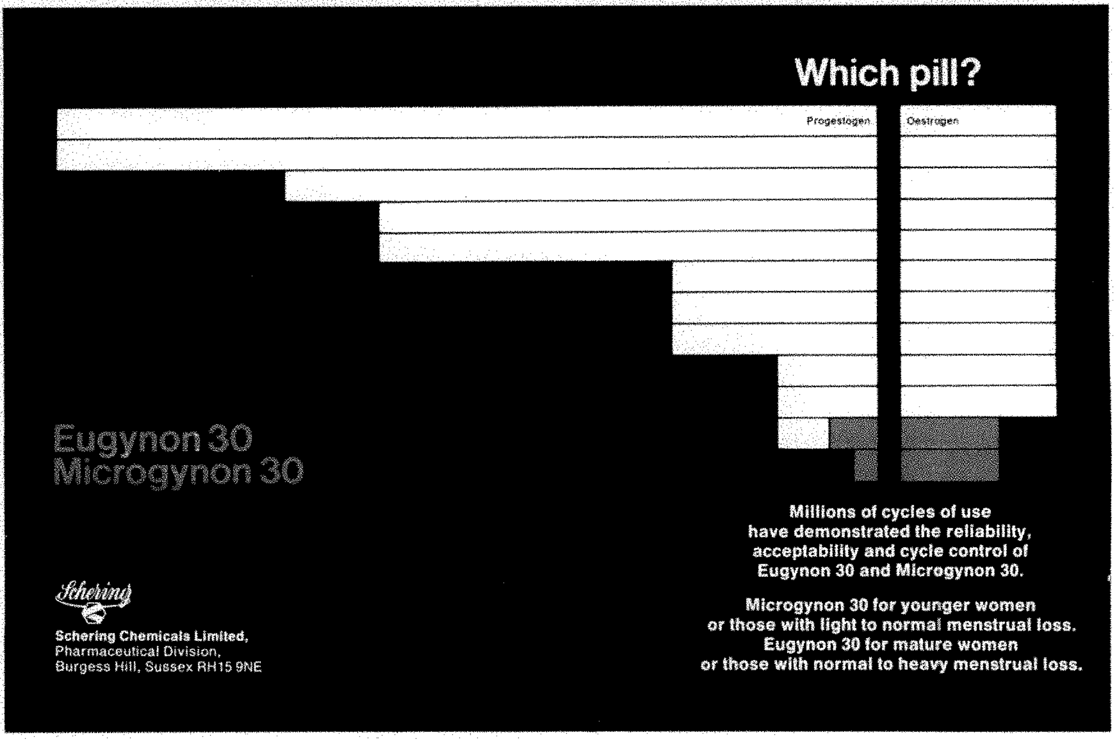

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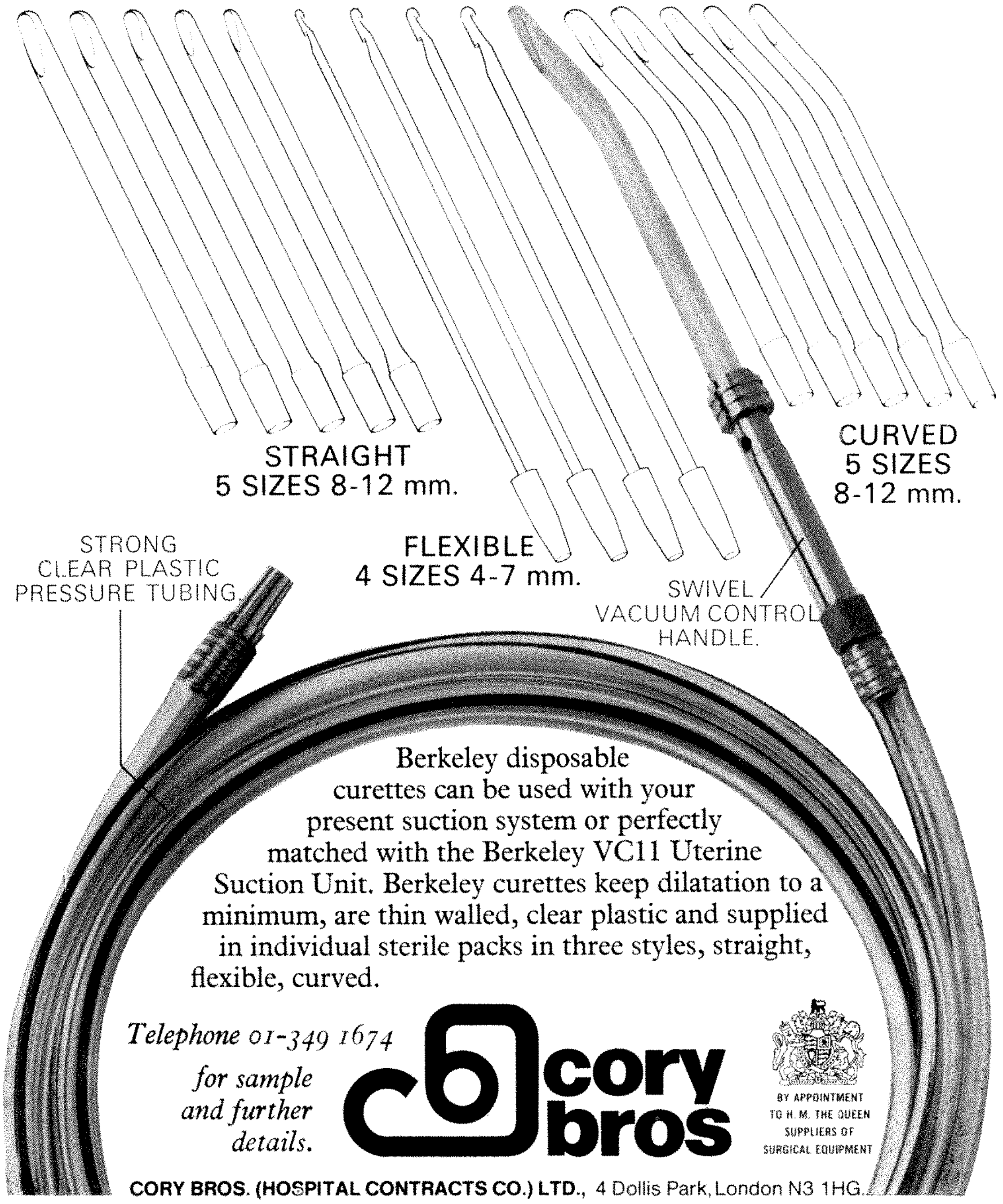

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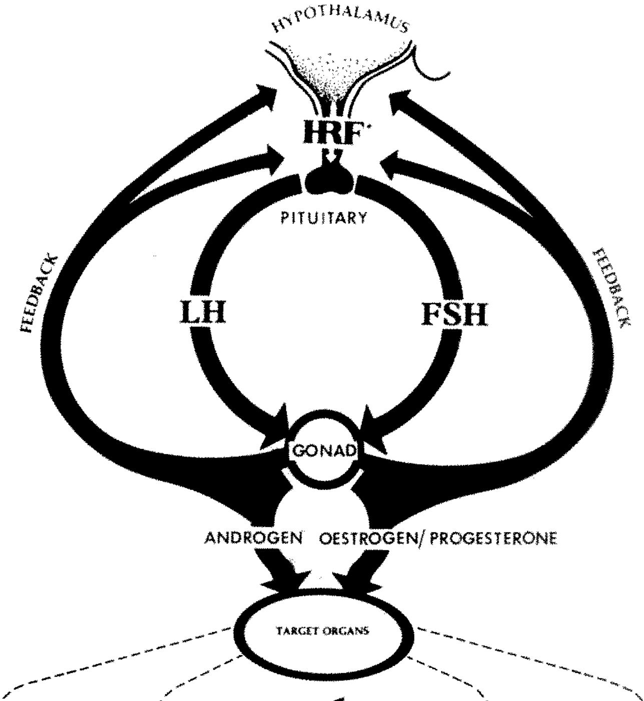

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**Female**
Forbes-Albright syndrome
Sheehan's syndrome
Stein-Leventhal syndrome
Hypothalamic organic
  lesions
Hypogonadotrophic
    hypogonadism
  Primary amenorrhoea
  Secondary amenorrhoea
  —without galactorrhoea
    anorexia nervosa
    iatrogenic
    idiopathic
    psychogenic
    post-traumatic
    postpartum
  -with galactorrhoea
    iatrogenic
    idiopathic
    non-postpartum
    postpartum

⚥

**Male and Female**
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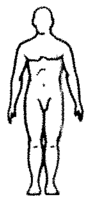
Cushing's syndrome
Prader-Willi syndrome
Simmonds' syndrome
Hypophysectomy
Pituitary ablation
Hypothalamic disorders
  Tumours
  Congenital lesions
    Kallmann's syndrome
Functional disorders
Panhypopituitarism
  Primary
  Secondary
    idiopathic or other
Hypogonadotrophic
  hypopituitarism
  Adult
  Pubertal



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Frohlich's syndrome
Klinefelter's syndrome
Hypogonadotrophic
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  Primary
    Cryptorchism
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**When the menstrual cycle is disturbed**

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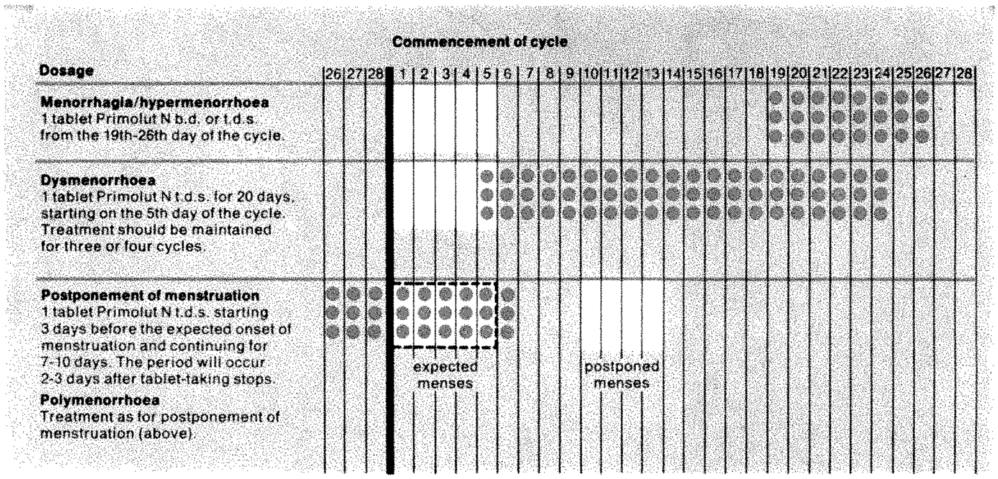

Commencement of cycle

| Dosage | 26 | 27 | 28 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Menorrhagia/hypermenorrhoea**<br>1 tablet Primolut N b.d. or t.d.s. from the 19th-26th day of the cycle. | | | | | | | | | | | | | | | | | | | | | | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | | |
| **Dysmenorrhoea**<br>1 tablet Primolut N t.d.s. for 20 days, starting on the 5th day of the cycle. Treatment should be maintained for three or four cycles. | | | | | | | | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | | | | |
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British Journal of Obstetrics and Gynaecology

VOL. 82 No. 2 NEW SERIES FEBRUARY 1975

# THE UTERINE CERVIX FROM ADOLESCENCE TO THE MENOPAUSE

BY

ALBERT SINGER*

*Nuffield Department of Obstetrics and Gynaecology*
*University of Oxford, Oxford*

## Summary

During adolescence the physiological transformation zone of the cervix in the virgin undergoes limited change when compared to that of girls who are sexually promiscuous; the latter often show large areas of metaplastic squamous epithelium and the development of an atypical transformation zone. During pregnancy, particularly the first, the transformation zone increases in size and acquires increased amounts of squamous metaplastic epithelium, probably due to the effect of acidic vaginal fluid on endocervical columnar epithelium. Evidence exists for the development of an atypical transformation zone during pregnancy with its own variable neoplastic potential. After delivery the atypical epithelium of the cervix may be partly or completely removed with resulting regression of the lesion. Alternatively, neoplastic potential may alter or the lesion may remain static or recur in its previous form. After the childbearing era the physiological transformation zone undergoes minimal change until the menopause when the whole cervix shrinks. As the studies described above dramatically show, the sexually promiscuous woman at all ages has an increased risk of developing cervical neoplasia; this risk seems to be highest in the third decade of life.

THE accessibility of the uterine cervix facilitates the study of its physiology and pathology. Not surprisingly, therefore, exfoliative cervical cytology achieved such universal acceptance which in turn led to intense study of the premalignant cervical epithelial changes of dysplasia, carcinoma *in situ*, and microcarcinoma. During the last three decades, especially in the U.K. and North America, the detection and ultimate confirmation of these premalignant lesions has relied almost exclusively on exfoliative cytology and histopathology. However, in Europe, the Americas and Australia, there has existed a third diagnostic discipline, namely, colposcopy. The illuminated and magnified image provided by the colposcope allowed the gap that existed between observations derived by naked eye study and those from histology to be filled.

Colposcopy principally amongst the three diagnostic disciplines enables precise study to be made of the continual changes that occur in the morphology of the cervix during the life of the sexually mature woman. The factors contributing to these alterations are physiological and pathological. Each produces a specific effect and contributes to the overall pattern of change undergone by the cervix. A chronological review of these changes from adolescence to the menopause is presented. The patients studied were from the University Departments of Obstetrics and Gynaecology in Sydney, Australia, and Oxford, England, and from a large prison in

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* Present address: University Department of Obstetrics and Gynaecology, Jessop Hospital, Sheffield S3 7RE.

Presented as the 47th Blair-Bell Memorial Lecture, 14th March, 1972.

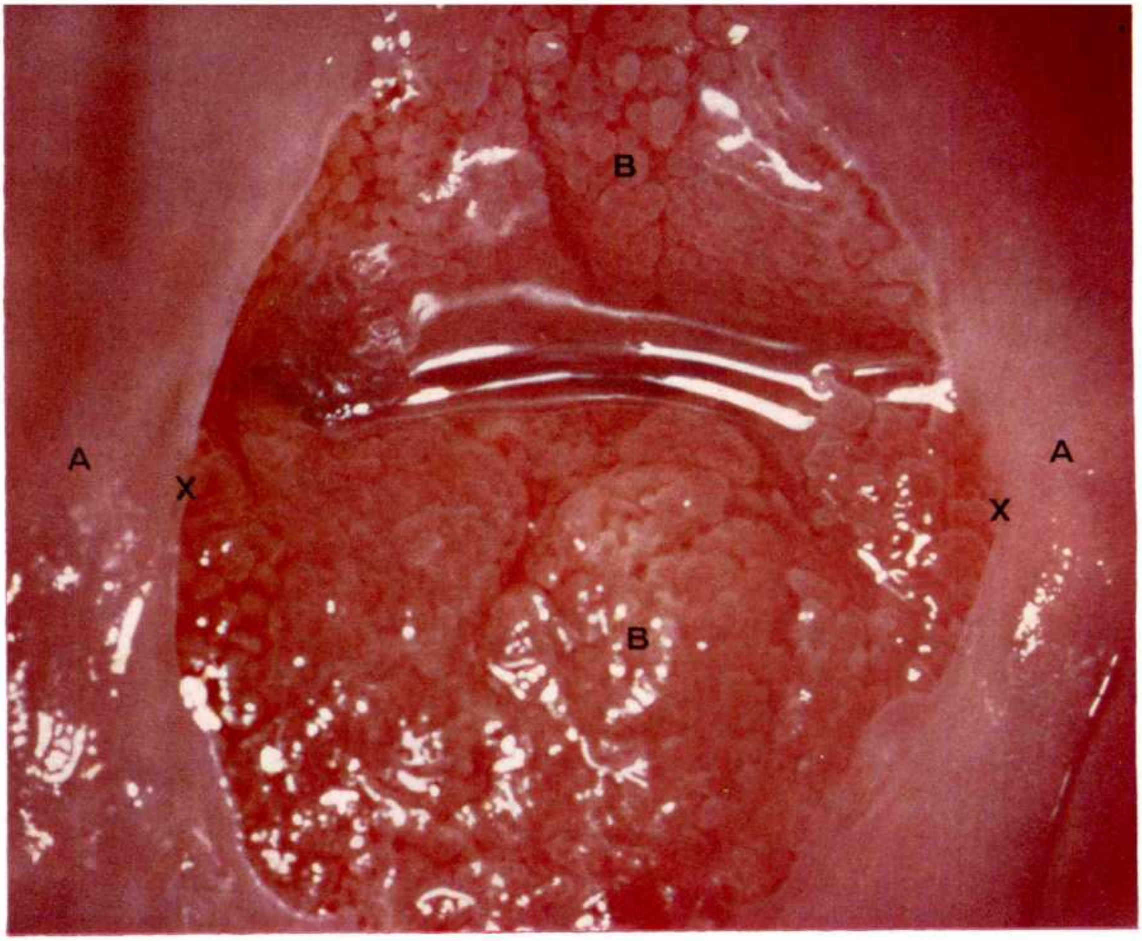

FIG. 1
Colpophotograph (×25) showing the two native types of epithelium, namely native squamous at A and native columnar at B. The squamocolumnar junction is at X.

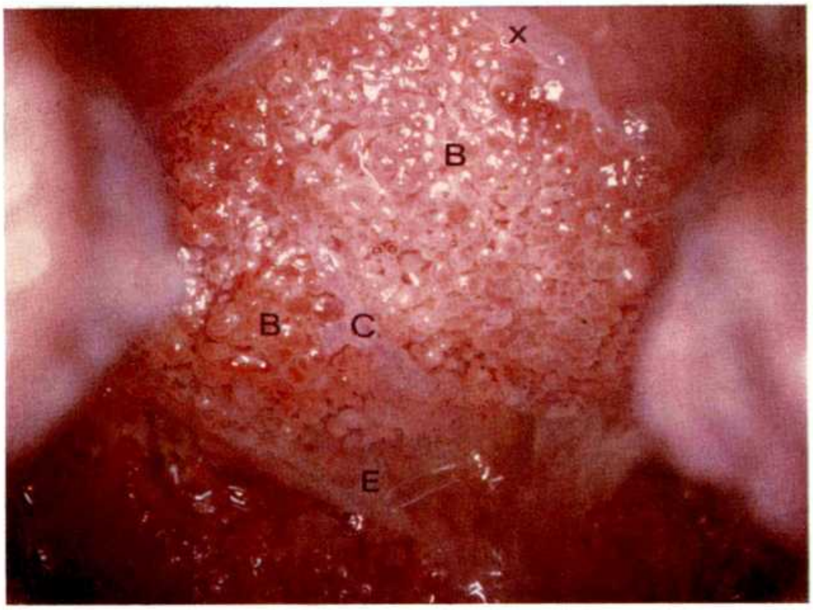

FIG. 2
Colpophotograph (×25) of the anterior lip of the cervix in a primigravida at 12 weeks of pregnancy. An area of squamous metaplastic epithelium (C) had developed within the area of the native columnar epithelium (B). The endocervical canal is at point E.

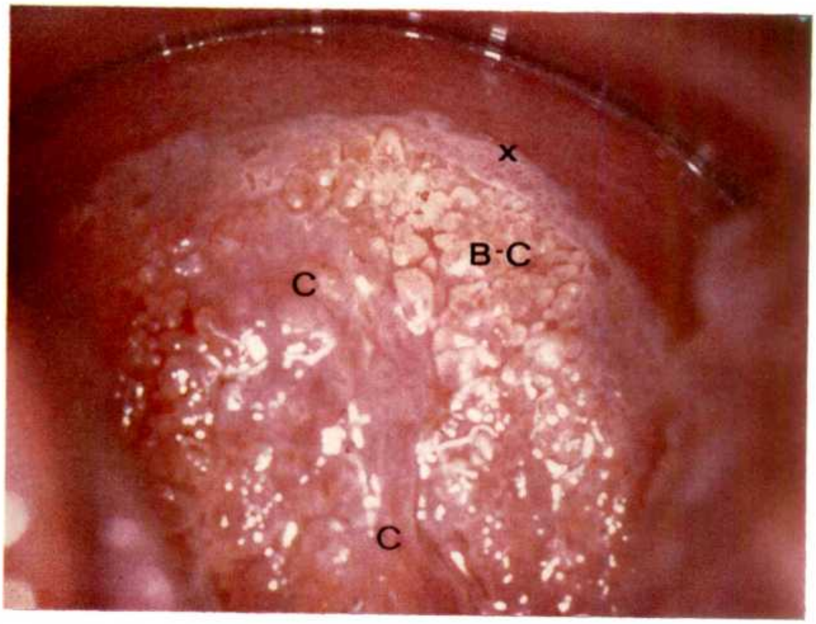

FIG. 3

Colpophotograph (× 30) at 36 weeks of pregnancy of the cervix shown in Figure 2. Fusion of adjacent villi of columnar epithelium has resulted in the formation of a smooth area of squamous metaplastic epithelium (C). Some of the villi undergoing fusion are seen in area B–C.

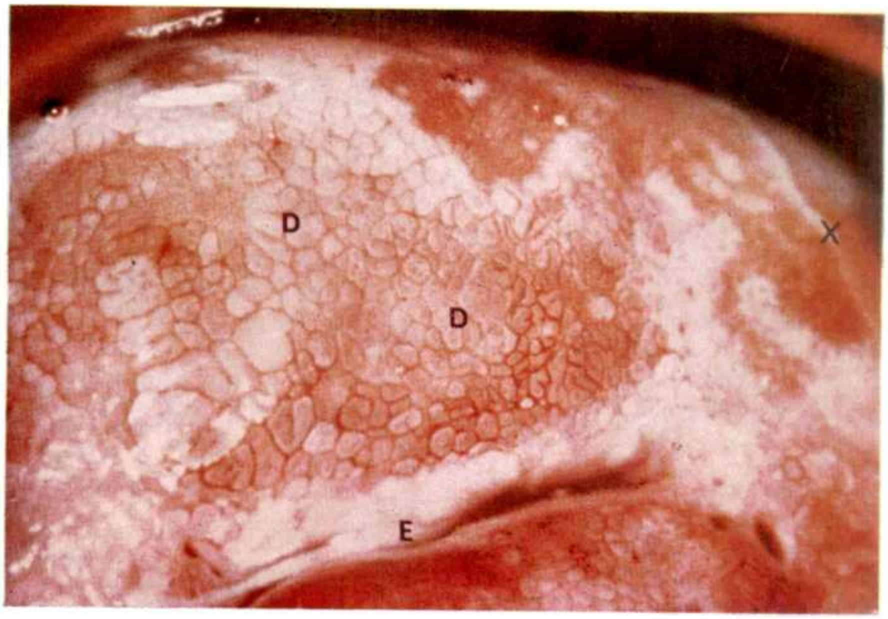

FIG. 4

Colpophotograph (× 20) of an atypical transformation zone. The atypical epithelium (D) appears white as a result of obstruction to reflected light and also possesses a mosaic tiled appearance consequent upon the presence of epithelial capillaries. Punch biopsy of this zone revealed histological evidence of carcinoma *in situ*. The squamocolumnar junction (X) is on the ectocervix while the endocervical canal is at point E.

the U.K. The colposcope used in most of the studies mentioned was the Carl Zeiss (Oberkocken) Colposcope Model I with in-built co-axial illumination and a rotating 5-step magnification drum. Colpophotographs were taken using the Contax camera attached to the Zeiss colposcope. A shutter speed of 1/50 of a second with an aperture of *f*32-45 was employed. The film used for colour photography was Kodachrome II, and for black and white, ADOX KB14.

## COLPOSCOPIC REPRESENTATION OF CERVICAL EPITHELIUM

To the colposcopist differentiation between normal and abnormal cervical epithelium is obvious as each specific *type* of epithelium has a characteristic and easily recognizable appearance. There are three such types, two of a purely *physiological* nature and a third with *pathological* associations.

The two physiological types are: (*a*) native epithelium which can be squamous or columnar, and (*b*) metaplastic squamous epithelium occurring in the area of the native columnar. The third type comprises pathological epithelia that are grouped together under the title of atypical epithelium and included are some with a neoplastic potential which *may* be expressed in the development of invasive carcinoma (Reid, 1971; Coppleson *et al.*, 1971*a*; Kolstad and Stafl, 1972*a*).

The native epithelia are originally laid down during intrauterine development and persist into adult life. The native squamous epithelium lines the vagina and is continued upwards to cover variable amounts of the cervical surface. Here it comes into contact with native columnar epithelium that is a downwards extension of the endometrial lining. The boundary occurs at a distinct, easily recognizable point, called the squamocolumnar junction (see Fig. 1). The position of this permanent junction may be placed at any point across the ecto- or endocervix. In 4 per cent of women it occurs in the vaginal fornix (Kolstad, 1970).

During certain periods of female life, alterations occur within the native columnar epithelium and result in the formation of the second type of epithelium called metaplastic squamous. The process of metaplasia occurs principally during pregnancy and adolescence and to a lesser degree during late intrauterine development; it is a physiological process occupying a short period measured in days and weeks (Coppleson and Reid, 1967*a*). The colposcopist regards the area medial to the squamocolumnar junction and occupied by native columnar epithelium in which metaplasia may occur as the typical or physiological transformation zone (Figs. 2 and 3).

There is evidence to suggest that the epithelium during the early stages of metaplasia is vulnerable to genetic change, resulting in its containing a cell population that has somehow acquired a neoplastic potential (Reagan and Patten, 1962; Johnson *et al.*, 1964; Reid, 1971). This, the third type of epithelium, has distinctive morphological characteristics possessing the same topographical arrangement within the transformation zone as the physiological epithelium. This transformation zone is called by the colposcopist, an atypical transformation zone (Fig. 4) and within its area may reside the precursors of squamous cervical carcinoma (Coppleson and Reid, 1967*b*; Kolstad 1970; Kolstad and Stafl, 1972*a*).

On colposcopy, atypical epithelium appears white as a result of the obstruction to reflected light by an increase in cellular density. It may also have a honeycombed or stippled appearance resulting from the shape of the epithelial capillaries as seen in Figure 4. The atypical transformation zone is graded colposcopically according to the degrees of difference in certain morphological features such as epithelial whiteness, vessel irregularity, and surface contour. Correlation is therefore possible between colposcopy and histology and is precise when major epithelial lesions such as severe dysplasia carcinoma *in situ* or microcarcinoma exist (Coppleson *et al.*, 1971*a*; Kolstad and Stafl, 1972*b*). Occasionally, correlation between the minor degrees of colposcopic and pathological abnormality is difficult. Accurate biopsy of atypical epithelium is possible under colposcopic vision.

In the studies to be described, extensive use was made not only of sequential colpophotography to monitor accurately the epithelial changes, but also of a method which measured the extent and distribution of individual epithelial types and allowed subsequent comparison

(Fig. 5). The method involved the projection of a colpophotograph onto graph paper with planimetric measurement of the area occupied by the individual epithelia from the contour map. The colpophotograph was only taken with the self-retaining Cusco vaginal speculum fully opened so that a complete view of both endo- and ecto-cervix was obtained (see Figs. 6 and 7).

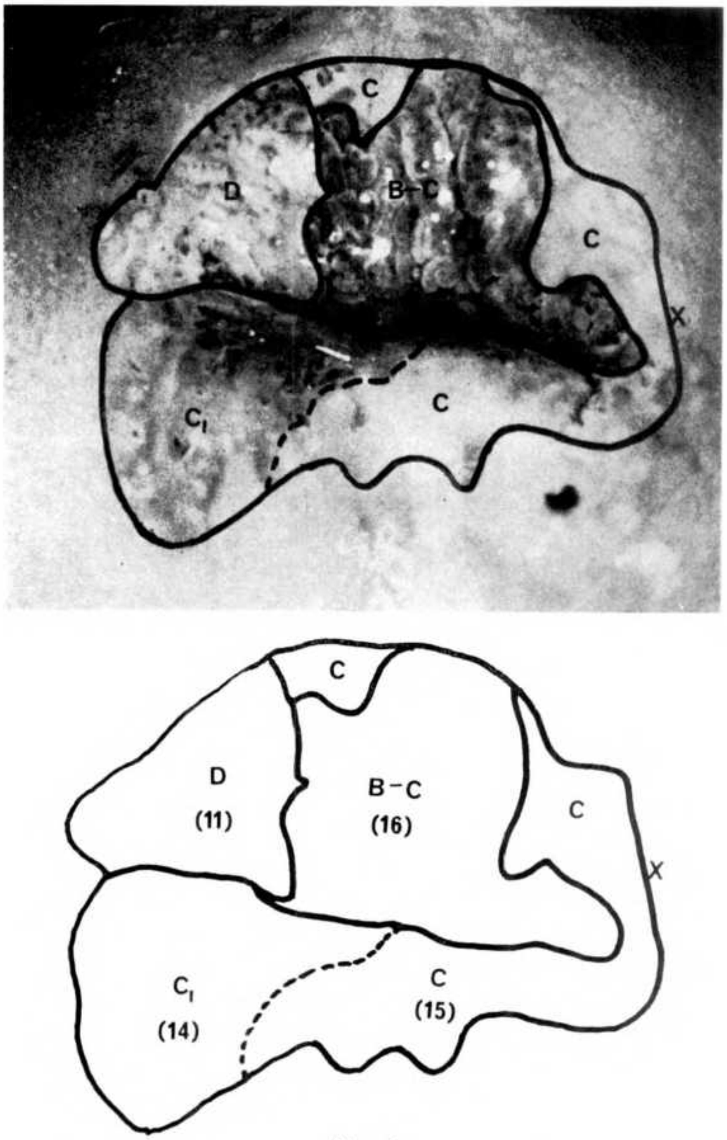

FIG. 5

Colpophotograph (× 6) with corresponding contour area map of an atypical transformation zone. Planimeter measurement has determined the relative areas of each individual type present. (B–C represents columnar epithelium with early metaplastic change, $C_1$ = recently formed metaplastic squamous epithelium, C = mature metaplastic squamous epithelium and D = atypical epithelium).

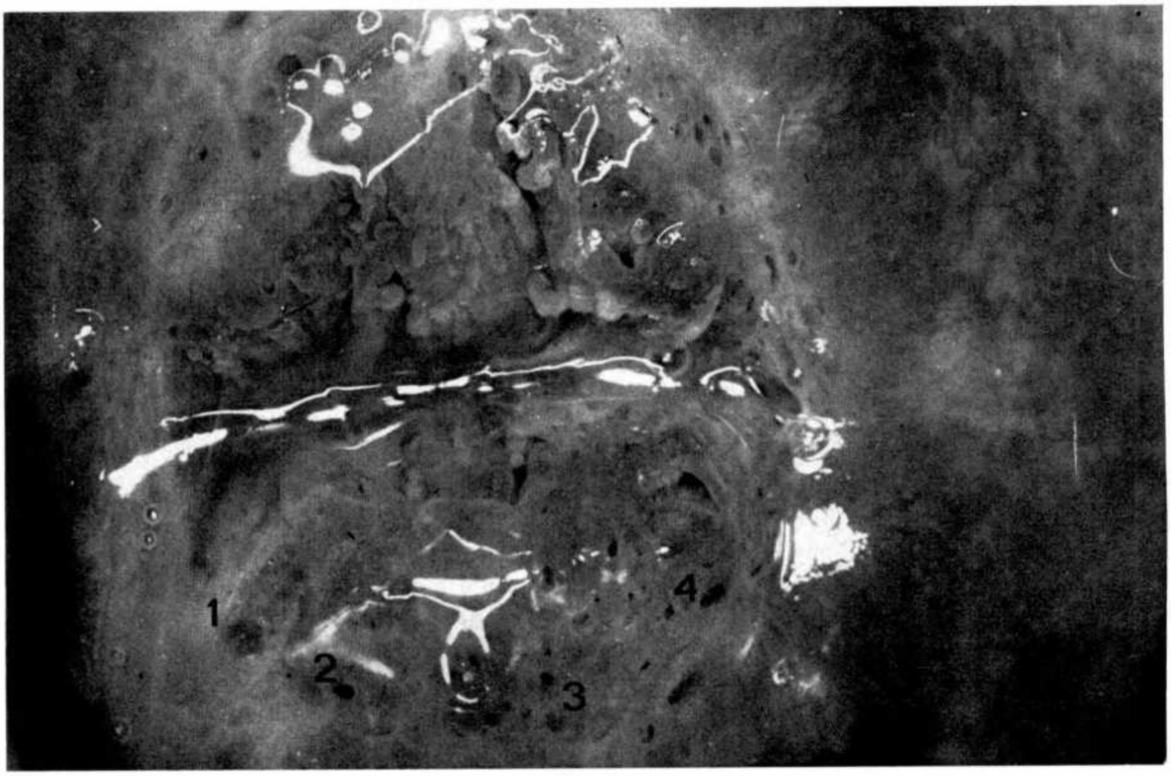

FIG. 6
Colpophotograph (× 10) of a physiological transformation zone taken with the Cusco self-retaining speculum fully opened and called the "apparent view". Points 1–4 show gland openings within metaplastic squamous epithelium (see Fig. 7).

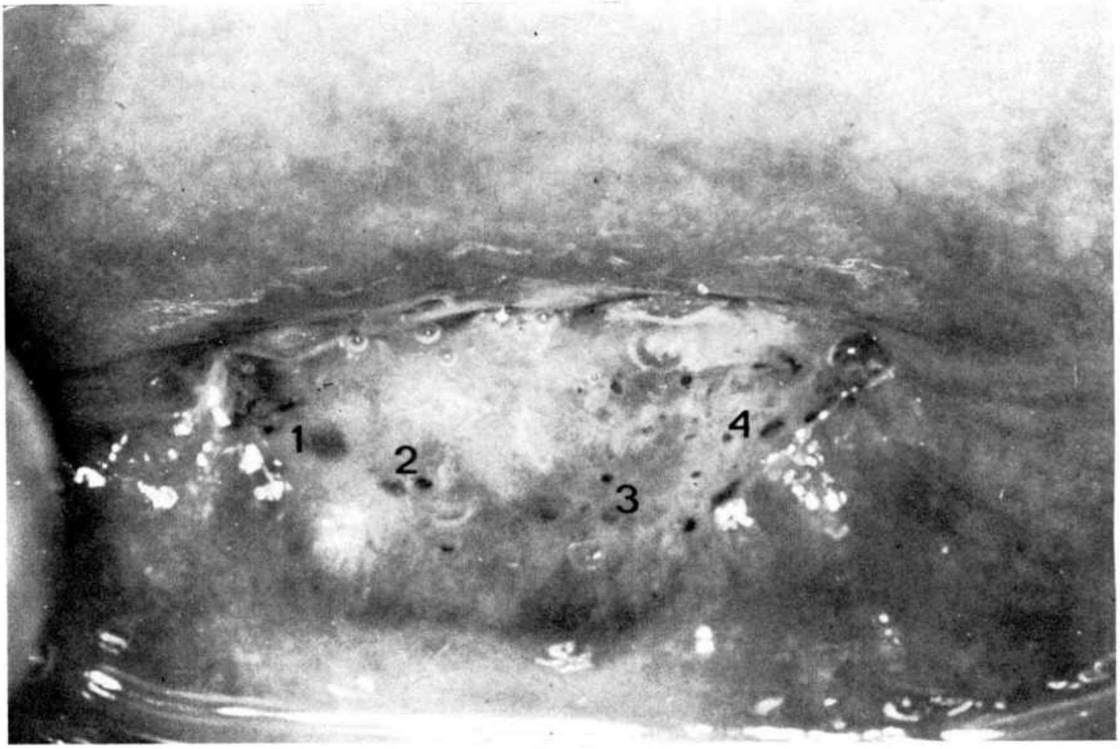

FIG. 7
Colpophotograph of the same cervix shown in Figure 6 now exposed by a partly closed Cusco's speculum which is withdrawn into the mid vagina. This view imitates the natural *in vivo* position of the cervical epithelium and is called by the colposcopist the "real view".

### The Cervix During Adolescence

The insistence by epidemiologists on the significance of adolescent intercourse and promiscuous sexual behaviour in the genesis of cervical carcinoma (Rotkin, 1967; Wynder, 1969) prompted the undertaking of two colposcopic studies to determine the effect of this form of behaviour on the adolescent cervix. In Sydney, Coppleson *et al.* (1972) colposcoped 300 sexually promiscuous and nulliparous girls who were under 17 years of age, detained in a state institution, who were by law obliged to undergo cytological and venereological examinations. In the second study within a large female prison in London, Singer (1973) colposcoped 150 nulliparous women under 21. In both studies information relating to behavioural characteristics was obtained by interview. The mean age of the Sydney group was 16·4 (±2·4 years) and of the London girls 18·5 (±2·2 years). First intercourse occurred at 13·4 (±2·7) years in the former and at 14·7 (±3·1) years in the latter group. Fourteen per cent of the Australian and 10 per cent of the London girls had coitus prior to the menarche.

Forty sexually mature virginal patients with a mean age of 19 had a colposcopy. They were admitted to hospital for examination under anaesthesia for such conditions as dysmenorrhoea, rigid hymen or dysfunctional uterine bleeding. Colpophotographs (with measurements of each individual epithelial type) of the cervices of 170 randomly selected promiscuous girls from the Sydney study and the 40 virginal girls were compared (Singer, 1972). The differences in morphological appearance as assessed by this technique between the two groups are shown in Table I.

The total area occupied by the transformation zone of the virginal cervix was about 60 per cent larger than that of the promiscuous girl. This difference was significant ($p=<0{\cdot}01$). This could be due to coitus causing small injuries or the contraction of uterine muscle connection with apparent shrinkage of the area of the transformation zone. Masters and Johnson (1966) and Goodlin and Shundt (1972) have shown an increase in uterine muscle activity during coitus.

In many of the promiscuous girls the epithelium appeared to be undergoing atypical transformation in association with the development of squamous metaplastic epithelium (see Fig. 8). Both processes could have had similar initiating factors. Many authors have proposed that dysplasia and carcinoma *in situ* are forms of abnormal metaplasia (Kaufmann and Ober, 1959; De-Brux and Dupre-Fremont, 1960; Reagan and Patten, 1962), and suggest that mutagenesis occurs during metaplasia, Copple-

TABLE I

*Epithelial composition of the adolescent transformation zone as assessed by colposcopy*

| | | Total area (mm.$^2$) | Type of epithelium | | | |
|---|---|---|---|---|---|---|
| | | | Native columnar | Early squamous metaplasia* | Mature squamous metaplasia† | Atypical epithelium |
| *Virgins*<br>n = 40 | Mean | 170 | 90 | 51 | 27 | 2 |
| | Range | (112–240) | (45–137) | (32–73) | (10–52) | (0–3) |
| | Per cent | | 53 | 30 | 16 | 1 |
| *Sexually promiscuous girls*<br>n = 170 | Mean | 117 | 14 | 65 | 25 | 13 |
| | Range | (72–132) | (8–26) | (33–84) | (12–32) | (9–27) |
| | Per cent | | 12 | 56 | 21 | 11 |

* Early squamous metaplasia was diagnosed colposcopically when there was an increase in pallor and fusion of the native columnar villi and smooth opaque surfaces were seen either as tongues or islands of tissue.

† Mature metaplasia was diagnosed colposcopically when there was epithelium with a characteristic reddish hue and associated gland openings and cystic inclusions (Nabothian follicles).

The differences in the amount of native columnar epithelium and in the amount of early metaplastic epithelium between the two groups was significant ($p=<0{\cdot}01$).

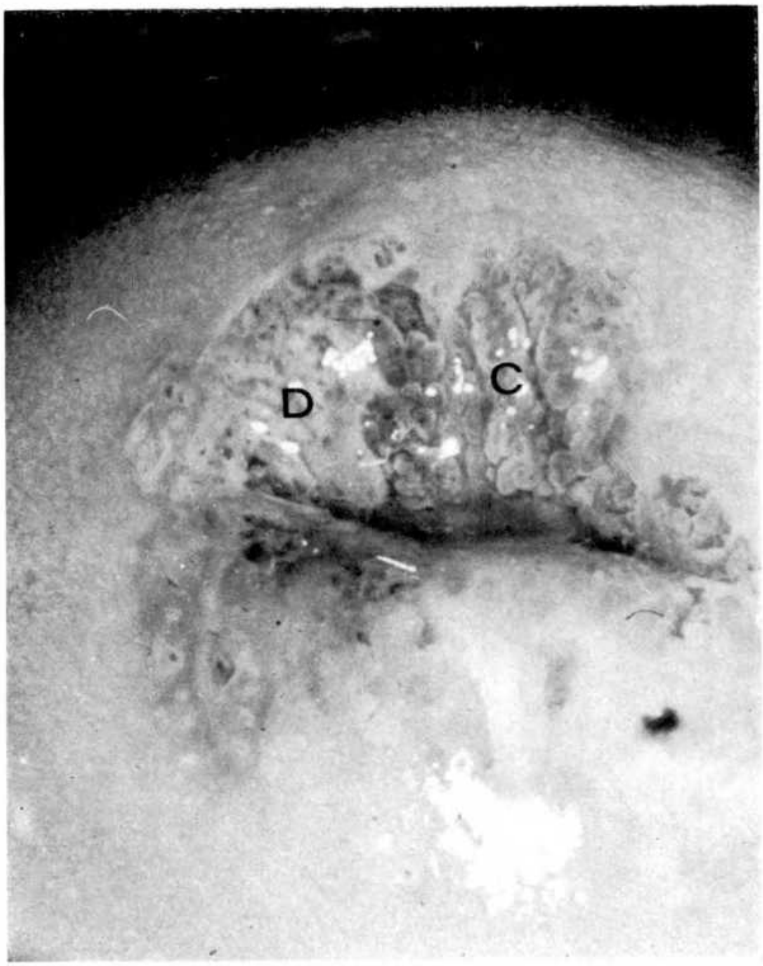

FIG. 8

Colpophotograph (× 10) of an atypical transformation zone in the cervix of a girl aged 15 showing the development of atypical epithelium (D) in close relationship to recently formed metaplastic squamous epithelium (C).

son and Reid (1967*a*) believe that the latter process may occur during the first pregnancy and adolescence.

Punch biopsies of the atypical transformation zone under colposcopic vision were taken from some of the Australian patients and from 90 per cent of those in the U.K. study. Precise correlation between colposcopic and histological abnormalities like severe dysplasia, and carcinoma *in situ*, was not always possible. This contrasts with the precise correlation that exists in adults between major colposcopic and histological abnormalities (Coppleson *et al.*, 1971*b*; Kolstad and Stafl, 1972*b*).

Evidence of some epithelial abnormality existed in 24 of the 70 punch biopsies taken from the atypical transformation zones that occurred within the cervices of 78 (52 per cent) of the London group of promiscuous girls. In 15 of them, lesions classified as abnormal epithelium (as described by Glatthaar, 1950) or basal cell hyperplasia, and considered to be benign (Govan *et al.*, 1966) existed. In nine patients, the youngest of whom was 17, dysplastic lesions, usually regarded as potentially malignant (Koss, 1968; Johnson *et al.*, 1968; Richart and Barron, 1969) were found. Five of the dysplasias were mild, while two were classified as moderate and two as severe. The remaining 46 of the 70 punch biopsies showed histological evidence of very active metaplastic epithelium with an associated intense inflammatory and "round cell" reaction involving the epithelium and the subepithelial areas. In view of the 65 per cent incidence of vaginal infection (cytologically or bacteriologically confirmed) the infiltration of the stroma and epithelium by mononuclear and lymphocytic cells rather than polymorphonuclear leucocytes may represent an immune reaction to a coitally transmitted antigen (Friedell *et al.*, 1960; Glucksmann and Cherry, 1964; Reid, 1971). The antigen may be of viral (Rawls and Gardner, 1972) or spermatozoal (Coppleson and Reid, 1967*c*) origin and the epithelium absorbing it may have neoplastic potential (Reid, 1971)

## THE CERVIX DURING PREGNANCY

By sequential colposcopy and colpophotography the changes in the cervix during pregnancy and labour were observed in 50 women, of whom 35 were primiparous. At each antenatal visit, colpophotographs were taken with the Cusco's speculum fully opened giving the "apparent view" (Fig. 6) and the speculum partly closed giving the "real view" (Fig. 7).

Study of colpophotographs showed that in the primiparous cervix during the first trimester, more columnar epithelium became exposed due to eversion of the endocervix (see Fig. 2).

In the second trimester, there was a further increase in the amount of eversion and a simultaneous increase in the area occupied by metaplastic squamous epithelium. The latter seemed to develop by *in situ* transformation and not by epithelial ingrowth from the squamocolumnar junction as had been proposed by Fluhmann (1959).

During the third trimester the islands of squamous epithelial metaplasia increased in size and fused with the squamocolumnar junction (see Fig. 3). The formation of large areas of metaplastic squamous epithelium occurred in 32 out of 35 of the primiparae studied. However, in only 10 out of 15 multiparae who were studied was there evidence suggesting the recent formation of metaplastic epithelium.

The multiparous cervix tended to gape rather than evert, with opening of the external cervical os. This occurred in 67 per cent of multiparous compared to only 20 per cent of primiparous cervices in this study and was noted from the 12th gestational week onwards, becoming most marked during the third trimester.

By employing the planimetric measurement technique the colposcopic observations described were quantified and the results are shown in Table II.

TABLE II

*The mean areas occupied by different epithelial types within the physiological transformation zone during each trimester of pregnancy as determined by the planimetric measurements of colpophotographs*

| Trimester | Mean area (mm.$^2$) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Primipara (35 patients) | | | | | | Multipara (15 patients) | | | | | |
| | Apparent view | | | Real view | | | Apparent view | | | Real view | | |
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Total transformation zone area | 146 | 184 | 176 | 65 | 76 | 110 | 160 | 173 | 220 | 89 | 93 | 125 |
| Columnar epithelium | 46 | 60 | 16 | 26 | 22 | 18 | 21 | 19 | 15 | 14 | 12 | 15 |
| Columnar epithelium with evidence of metaplastic change | 39 | 42 | 46 | 14 | 12 | 17 | 47 | 39 | 35 | 17 | 15 | 15 |
| Recently formed squamous metaplastic epithelium | 30 | 54 | 84 | 10 | 25 | 54 | 52 | 63 | 128 | 42 | 48 | 74 |
| Mature squamous metaplastic epithelium | 31 | 28 | 32 | 15 | 17 | 21 | 40 | 52 | 42 | 16 | 18 | 21 |

TABLE III

*The pH values at certain points on the cervix and on the endocervical canal during the first and third trimester of pregnancy and in the non-pregnant state*

| Position of pH electrode | | pH | | | | | |
|---|---|---|---|---|---|---|---|
| | | Non-pregnant (60 patients) | | Pregnant (35 patients) | | | |
| | | | | 1st Trimester | | 3rd Trimester | |
| | | Prolifera-tive phase Day 9–10 | Secretory phase Day 22–24 | Primiparae | Multiparae | Primiparae | Multiparae |
| Vaginal fornix | Mean | 4·6 | 4·4 | 4·3 | 4·3 | 4·3 | 4·3 |
| | Range | (3·3–7·0) | (3·6–6·0) | (3·4–5·4) | (3·4–5·4) | (3·4–5·9) | (3·4–5·9) |
| Extocervix: (at a point midway between vaginal fornix and external cervical os) | Mean | 5·7* | 5·6 | 4·7* | 4·9 | 4·8 | 4·8 |
| | Range | (3·9–7·2) | (4·3–7·0) | (3·7–5·3) | (3·7–5·5) | (3·8–6·5) | (3·8–6·6) |
| Endocervical canal | Mean | 6·8 | 6·1 | 6·7 | 6·4 | 6·9 | 6·1 |
| | Range | (5·5–8·0) | (5·1–8·4) | (5·4–7·7) | (5·4–7·2) | (4·1–8·0) | (4·1–7·8) |

* Values between which there is a significant difference ($P = <0{\cdot}01$).

The high incidence of metaplastic epithelial areas in positions exposed to the vaginal secretions suggested that they might be responsible for initiating metaplasia (Walz, 1958). The cervical and vaginal pH were therefore measured using fine direct-readout glass microelectrodes which had a 4 mm. measuring surface and were about 25 cm. in length (Philips C 146A type). These were placed at specific points at various stages during pregnancy and 35 women of whom 26 were primiparae were studied. In all, 134 measurements were made and the results are shown in Table III where they are compared with those obtained in 60 non-pregnant women (Singer, 1972). There was a significant drop ($p<0{\cdot}01$) in the pH of the vaginal secretions bathing the ectocervix during early pregnancy and this drop may indirectly stimulate metaplasia in columnar epithelium which becomes exposed by the eversion process of pregnancy to acidic vaginal secretions.

## EFFECT OF VAGINAL DELIVERY ON THE CERVIX

The passage of a fetus through the cervix caused the most dramatic and major alterations. Fifty women who had been examined by colposcopy throughout the antenatal period were re-examined in the puerperium. In addition, 125 consecutive patients were examined by colposcopy within two to five days of delivery. About half of these were multiparous.

Four specific lesions occurred on the cervix as a result of delivery and they were classified with the help of suggestions made by Wilbanks and Richart (1967) as: *ulceration* (an area recognized colposcopically and histologically by the absence of the original overlying cervical epithelium, Fig. 9); *laceration* (an area defined by a linear separation or tear within the epithelium and which extended into the stroma, Fig. 10); *bruising* (an area of subepithelial haemorrhagic discolouration which ranged from a small petechial haemorrhage to an area of gross contusion. and *yellow areas* (distinctly coloured tissue regions usually associated with the edges of deep laceration composed histologically of structureless necrotic tissue with inflammatory cell infiltration).

The cervix was involved by traumatic lesions in 72 per cent of primiparae and 52 per cent of multiparae. This difference was significant ($p<0{\cdot}01$). The anterior lip of the cervix tended to be damaged more often than the posterior lip being involved in 78 per cent of primiparae and in 61 per cent of multiparae compared to a respective involvement of the posterior lip of

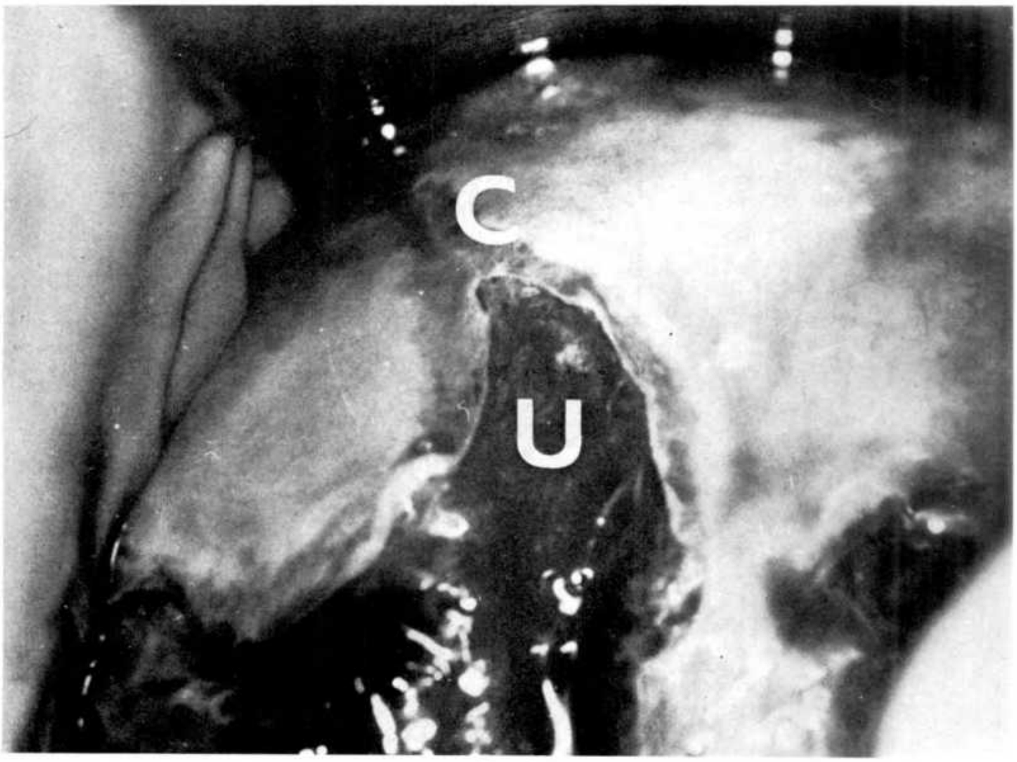

FIG. 9

Colpophotograph (× 20) of the anterior lip of the cervix in a primipara at 36 hours after a normal delivery. The epithelium is missing over a large area (U). Healing by metaplastic squamous epithelium (confirmed on biopsy) has already occurred over the upper part of this lesion (C) which is classed as an ulceration.

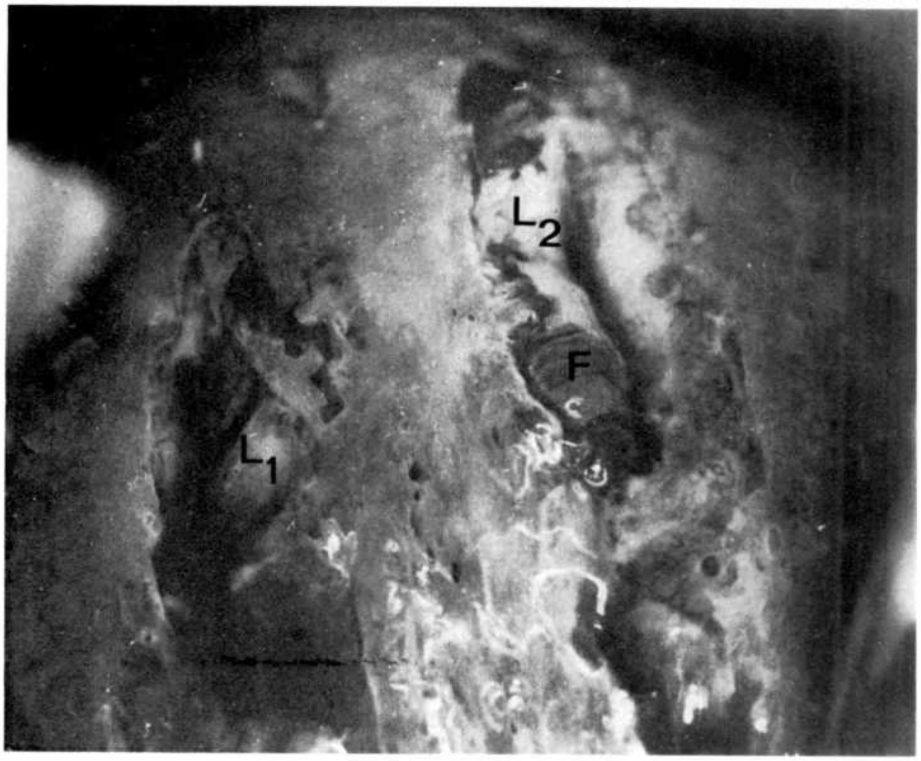

FIG. 10

Colpophotograph (× 20) of the anterior lip of the cervix in a primipara at 24 hours after a normal delivery. Two large *lacerations* (L1, L2) extending into the cervical stroma, radiate from the cervical canal. In laceration L2 a small Nabothian follicle (F) is visible.

66 per cent and 42 per cent. These differences were significant ($p<0 \cdot 05$). Ulcerations of the cervix were seen in 83 per cent of primiparae who had a traumatized cervix and in 72 per cent of multiparae with trauma to the cervix. They were significantly ($p<0 \cdot 01$) more common than lacerations which were the next most frequent lesions.

When a cervix showing ulceration or laceration was examined again 6 to 12 weeks later, it was uncommon to find any residual evidence of the lesion (see Figs. 11 and 12). Biopsies taken under colposcopic control during the healing process showed that within 36 hours a cellular monolayer and at 72 hours a multilayered covering had formed (Singer, 1972).

## DEVELOPMENT OF THE ATYPICAL TRANSFORMATION ZONE

Epidemiologists have insisted for some time that pregnancy is an important aetiological factor in the genesis of cervical carcinoma quoting as evidence its higher frequency in the parous compared to the nonparous woman (Wynder, 1969). During the colposcopic study already described of the cervix during pregnancy and the puerperium in 35 unselected primiparae the development of an atypical transformation zone was observed in seven of them. None developed in the multiparae. Histological evidence was obtained by punch biopsy from the atypical transformation zone in all women after the 16th week postpartum. One biopsy showed

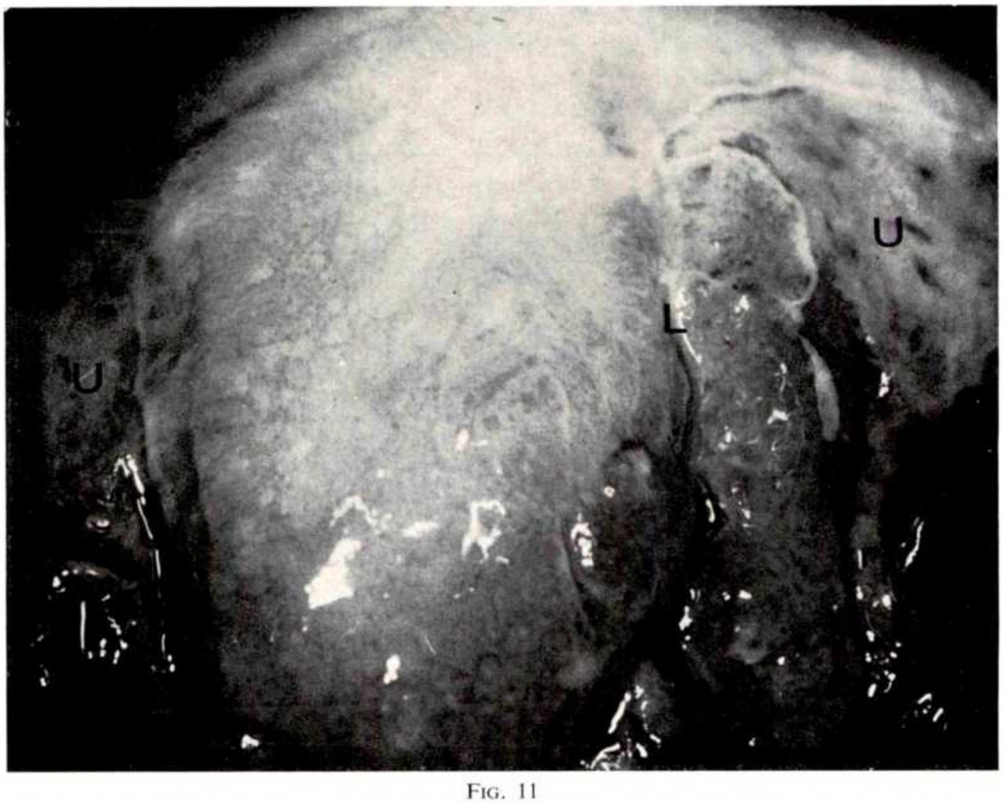

FIG. 11

Colpophotograph (×20) of the anterior lip of the cervix of a primipara at 2 days after a spontaneous delivery. Two ulcerations (U) and one laceration are seen. The traumatic lesions occurred with an atypical transformation zone which had been found six months prior to pregnancy and a biopsy at that time had shown moderate dysplasia. Dyskaryotic cells were present in a cytological smear at that time. The same area was examined again after 54 days and the results are shown in Figure 12.

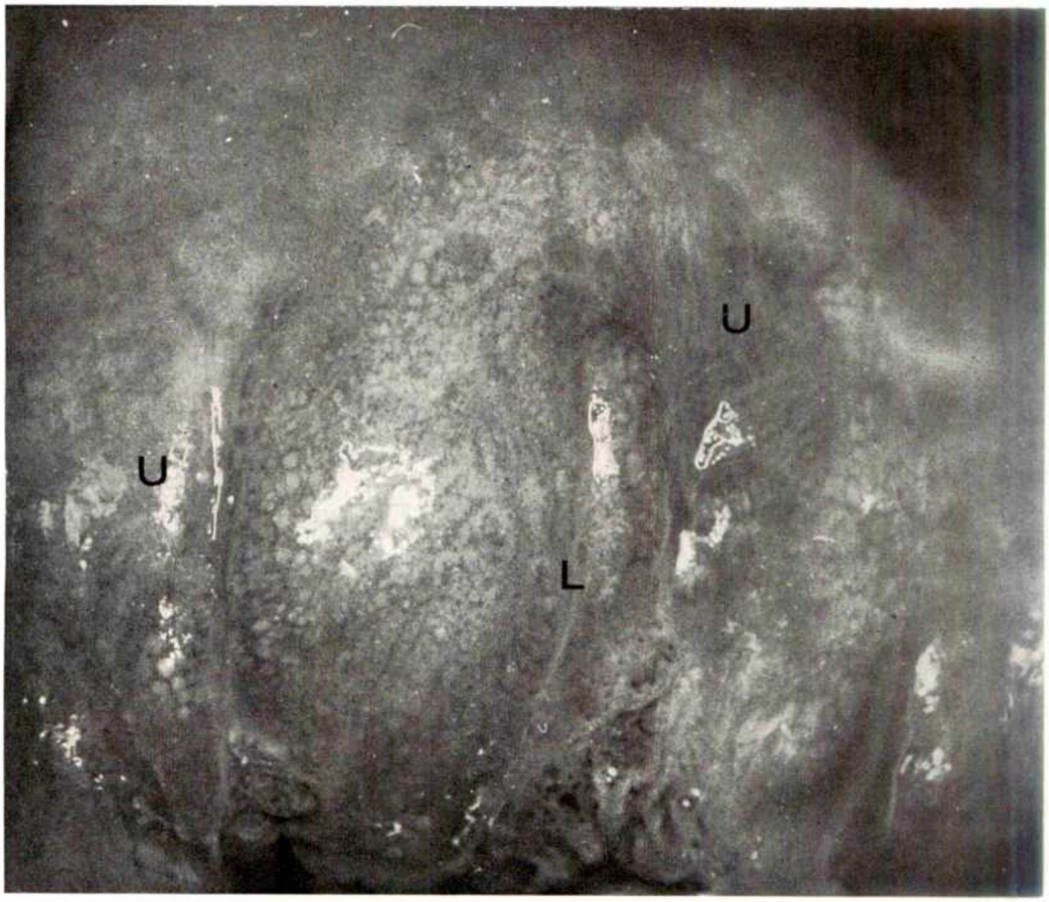

FIG. 12

Colpophotograph (×20) at 56 days postpartum of the same area of the anterior lip of the cervix as shown in Figure 11. Healing has occurred and the sites of laceration (L) and ulceration (U) are marked. Biopsies now showed only immature squamous metaplasia and a cytological smear was normal. It was thought that the atypical epithelium found previously had been removed by trauma at the time of delivery.

carcinoma *in situ*, and two showed severe dysplasia. Cervical cytology in each of the three major lesions was negative at the first antenatal visit but had changed to doubtful (Papanicolaou Class 3) in two patients and positive in one patient by the third month after delivery. In two further patients there was mild dysplasia and two patients had abnormal epithelium (Glatthaar, 1950). All four had negative cytological smears both before and shortly after delivery. Two of these patients were again examined by colposcopy two years later and the atypical transformation zone was still present; one patient now had a doubtful smear (Papanicolaou Class 3). The other two patients were lost to long-term follow-up.

Three patients first developed atypical epithelium at 26, 30 and 34 weeks of pregnancy. In the others it was first seen in the puerperium. Confirmatory punch biopsy of the atypical transformation zone was delayed until the fourth month postpartum in case spontaneous regression occurred. The series of colpophotographs (Figs. 13 to 16) show the development of one of these lesions in a 19-year-old primipara.

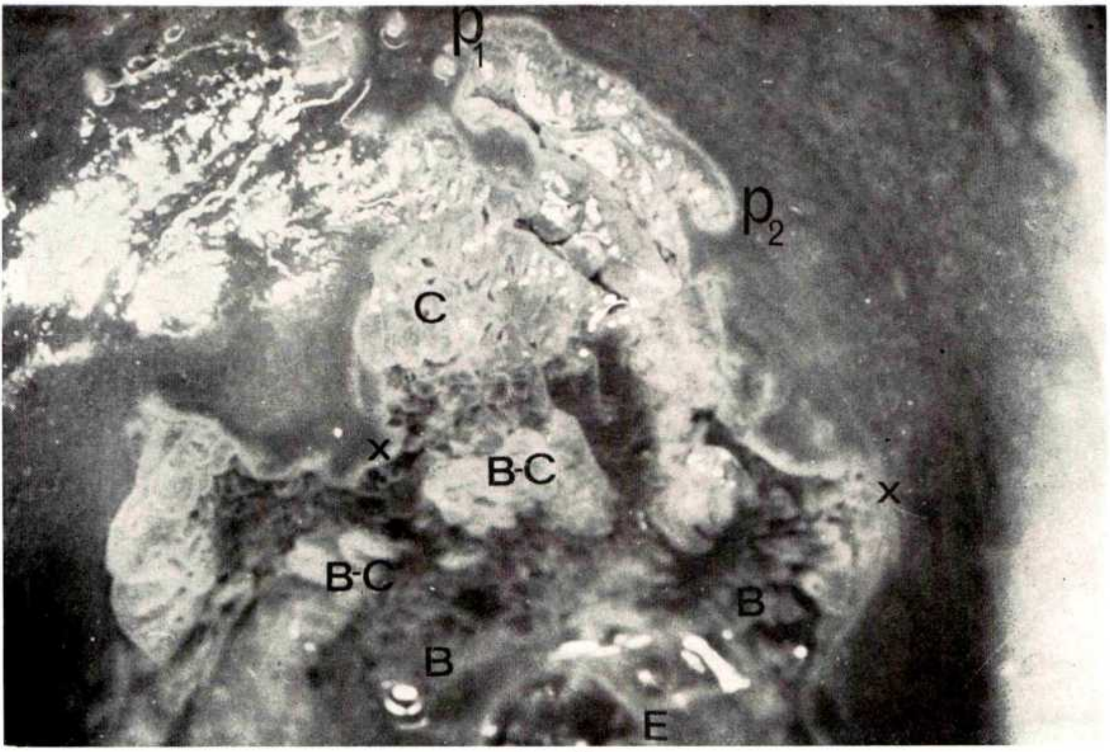

FIG. 13

Colpophotograph (×15) of the anterior lip of the cervix in a 19-year-old primipara at 12 weeks gestation showing columnar epithelium at point B and columnar epithelium with very early metaplastic transformation at points B–C. Early squamous metaplastic epithelium is at C and between $P_1$ and $P_2$. The squamocolumnar junction is at X and the endocervical canal is at point E.

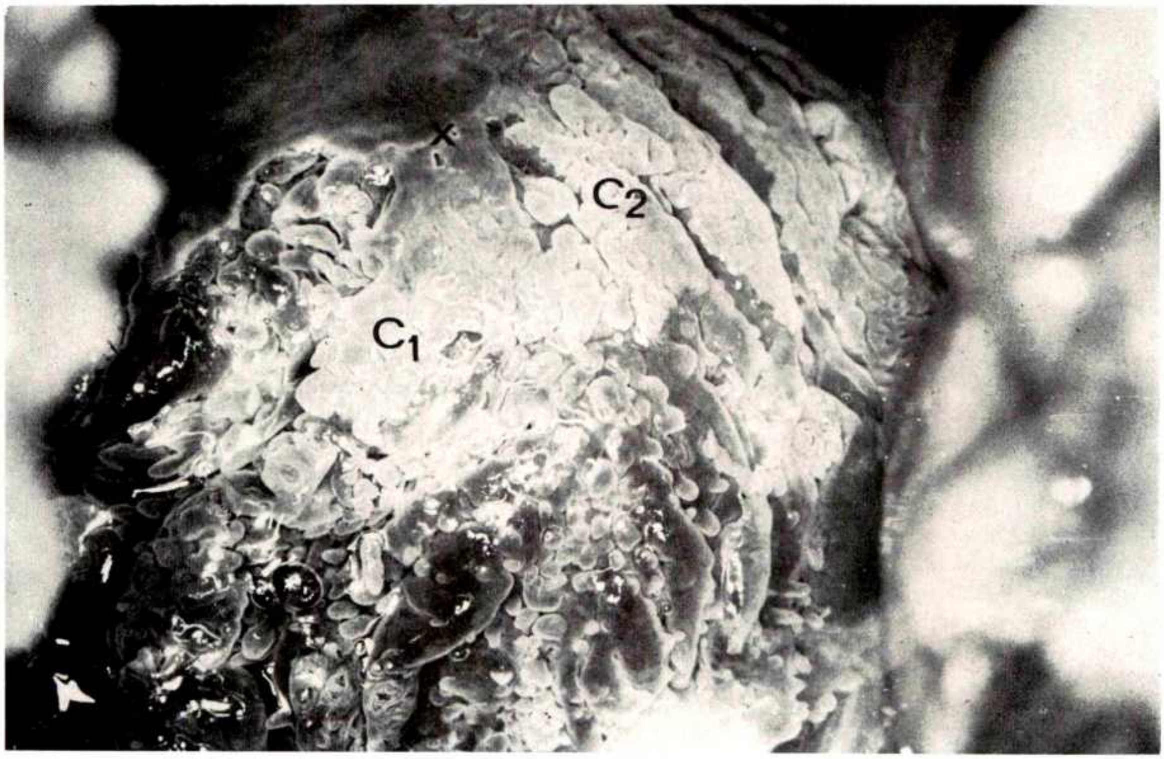

FIG. 14

Colpophotograph (×15) of the anterior lip of cervix shown in Figure 13 at 24 weeks gestation showing eversion of the anterior cervical lip and the previous islands of columnar epithelium (B–C in Fig. 13) having undergone metaplastic transformation to squamous epithelium ($C_1$ and $C_2$) and joining the original squamocolumnar junction. The whiteness between points $C_1$ and $C_2$ is artificial and caused by flashlight reflection.

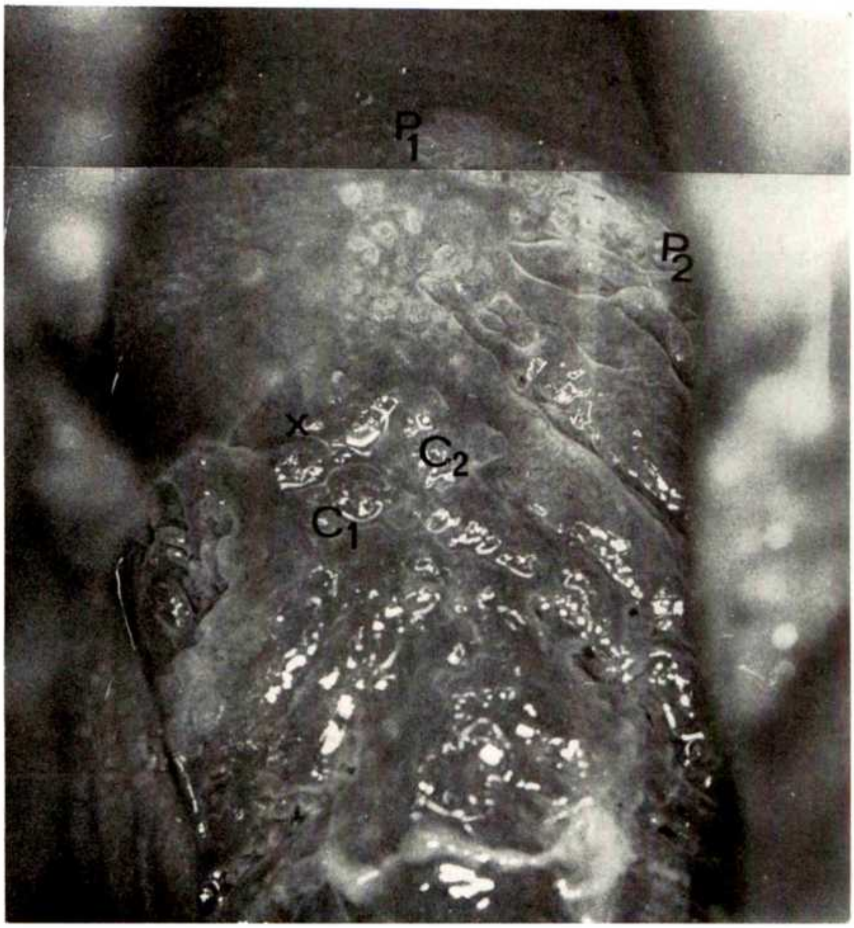

FIG. 15

Colpophotograph (×20) of the anterior lip of cervix shown in Figures 13 and 14 at 34 weeks gestation. Complete metaplastic transformation has occurred. A very faint abnormal vascular pattern exists between points $P_1$ and $P_2$. Points X, $C_1$ and $C_2$ are on the same points as in Figure 14.

### REGRESSION OF THE ATYPICAL TRANSFORMATION ZONE

It has been suggested by many authors (Marsh, 1956; McKay *et al.*, 1959) that regression during pregnancy of dysplasia and carcinoma *in situ* was common, and regression rates using cytological criteria, ranging from 20 to 75 per cent, have been quoted (Reagan, 1964).

The trauma to the cervix at the time of delivery provided a mechanism for the removal of atypical epithelium. During the studies described previously, many instances were encountered in which this occurred (see Figs. 11 and 12).

### EFFECT OF AGE ON THE CERVIX

It had been an impression that the cervix underwent little change after the childbearing period. Multiple colpophotographs taken over periods of up to 12 years by Coppleson and Reid (1967*d*) of the cervix of premenopausal women who had completed their families, showed little morphological change. This suggested to them that age did not influence the morphology of the cervix.

To investigate these impressions further, 350 consecutive women between 25 and 65 years of age were examined by colposcopy and the

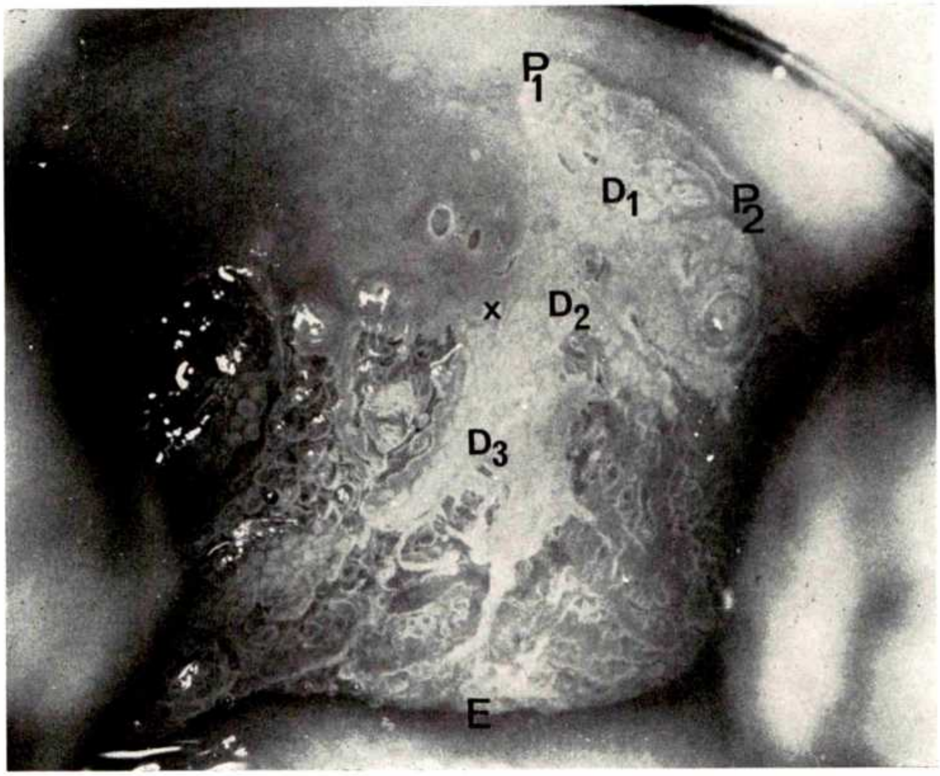

FIG. 16

Colpophotograph (×25) of the anterior lip of cervix as shown in Figures 13, 14 and 15 at six weeks after delivery. Atypical epithelium ($D_1$–$D_3$) extends longitudinally towards the endocervix. This epithelium exists in an area that was originally occupied by columnar and then by metaplastic squamous epithelium. Biopsy at multiple points across this longitudinal atypical area ($D_1$–$D_3$) showed severe dysplasia. Points X, $P_1$ and $P_2$ correspond with the similarly labelled points in Figure 15.

TABLE IV

*The effect of age on the relative area occupied by individual epithelial types within the transformation zone, as visualized by colposcopy in the apparent and real views. The area occupied was determined using the planimetric measurement of colpophotographs* (*Coppleson and Reid*, 1967c)

| Age | No. of viable pregnancies | | Fraction of total area occupied by specified epithelium (per cent) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Apparent view | | | | Real view | | | |
| | Mean | SD | Columnar epithelium | Early or recent metaplasia change | Mature squamous epithelium | Atypical epithelium | Columnar epithelium | Early or recent metaplasia change | Mature squamous epithelium | Atypical epithelium |
| 21–30 (n = 50) | 1·7* | 0·8 | 30 | 28 | 29 | 13 | 17 | 20 | 42 | 21 |
| 31–40 (n = 82) | 2·8* | 1·1 | 18 | 14 | 58 | 10 | 9 | 7 | 71 | 13 |
| 41–65 (n = 68) | 2·5 | 0·9 | 10 | 11 | 67 | 12 | 4 | 6 | 77 | 13 |

* Values between which there is a significant difference ($p = < 0{\cdot}01$).

distribution of epithelial patterns measured by the planimetric technique previously described. The results are shown in Table IV and are also described elsewhere (Coppleson and Reid, 1967*d*; Singer, 1972). The major changes occurred between the second and third decades of life. These changes seemed to be related to the occurrence of pregnancy and not to increasing age. The differences between the colposcopic appearance of the cervix in the third decade and the fourth and subsequent decades were mainly due to increasing maturity of metaplastic squamous epithelium which is recognized by the development of Nabothian follicles and the increasing vascularity of the epithelium. There was also a gradual but not significant decrease in the overall size of the transformation zone in postmenopausal women, with subsequent retraction of it into the endocervical canal.

## EFFECT OF PROMISCUOUS SEXUAL BEHAVIOUR ON THE CERVIX

Although parity seems to be the major determinant of cervical morphology, promiscuous sexual behaviour could further influence the transformation zone. Reference has been made previously to its effect on the adolescent cervix where it was associated with an increase in the amount of metaplastic and atypical epithelium.

A total of 300 women between the ages of 21 and 50 was studied. The pattern of their sex life can be judged by their age at first sexual intercourse (mean 16·4±2·5 years), the number of sexual partners (mean 4·6±1·7 in the first three years after commencing coitus), and a venereal disease rate of 45 per cent.

These women were confined to a large U.K. female prison and were examined by colposcopic, cytological and histological methods. A punch biopsy was taken of every atypical transformation zone seen and this ensured that a true histological prevalence rate for epithelial abnormalities was achieved.

In Table V preliminary findings relating to the prevalence rate for the colposcopic, cytological and histological diagnosis of epithelial abnormalities as detected in this study, are compared with rates obtained from the literature as summarized by Gray (1964), Coppleson and Reid (1967*e*) and Stern (1969). The differences between the two groups is striking. The abnormal colposcopy rate refers to the presence of an atypical transformation zone. Major histological abnormalities refer to those lesions diagnosed as dysplasia (mild, moderate, severe) or carcinoma *in situ*. Their frequency can be seen in Table VI.

TABLE V

*Frequency and prevalence rates for epithelial abnormalities as detected by colposcopy, histology and cytology, for the prison population and for a general and representative group of other women*

| | Prison group London (age 21–50) (n = 300) | Non-prison groups* (Well women, gynaecological outpatients) |
|---|---|---|
| Overall abnormal *colposcopy* rate | 45 per cent | 5–15 per cent |
| Major *histological* abnormalities | 31/300 (103/1000) | 14–36/1000 |
| Major *cytological* abnormalities | 16/300 (53/1000) | 5–30/1000 |

* As summarized by Gray (1964), Coppleson and Reid (1967*e*) and Stern (1969).

Major cytological abnormalities refer to cervical smears classified as either positive (3 smears) or dyskaryotic (13 smears). In the latter group 9 abnormalities were of a major and 4 of a minor type. In all, 17 per cent of this population had never been screened, and in 18 per cent the smear was regarded as inflammatory and a repeat smear was indicated. In a further 2 per cent the specimen was unsatisfactory. Significant false negative and positive cytological smears occurred and are discussed elsewhere (Singer, 1973).

TABLE VI

*Frequency of major histological abnormalities within the prison population*

| | *n* = | Rate/1000 |
|---|---|---|
| Mild dysplasia | 10 | 33 |
| Moderate dysplasia | 7 | 23 |
| Severe dysplasia | 12 | 40 |
| Carcinoma *in situ* | 2 | 7 |
| | 31 | 103/1000 |

TABLE VII

*Frequency rate of major histological abnormalities by age within the prison population*

| Age | Total number in group | Number of patients with histological abnormality | Rate per 1000 |
|---|---|---|---|
| less than 21 | 150 | 9 | 60 |
| 21–25 | 130 | 12 | 81 |
| 26–30 | 81 | 12 | 148 |
| 31–40 | 59 | 5 | 85 |
| more than 40 | 30 | 2 | 60 |

When 150 adolescents were added to the study group and they were all divided into age groups, certain features emerged (Table VII). The incidence of major abnormalities showed a peak in the third decade after which it decreased.

Finally, a comparison was made between histological and colposcopic findings and certain aspects of sexual behaviour (Table VIII). All women were interviewed on two occasions by trained prison staff who had obtained their confidence. Information collected on both occasions was compared and a high rate of consistency existed. The data showed that women with physiological epithelium possessed features not markedly different from either one of two groups with proven histological abnormalities.

There was a group of distinctive histological lesions that are diagnosed histologically by other authors as basal cell hyperplasia (Gray, 1964) or abnormal epithelium (Glatthaar, 1950). They were classed as minor lesions and had an overall frequency of 143 per 1000. This frequency was not influenced by age. As these lesions tended to occur in women with unstable marital backgrounds who used no contraception and started coitus early, they may be the result of infection and coital trauma.

Major histological lesions tended to be most frequent in those single women, who had more than ten partners during their first three years of sexual life, who had a high illegitimacy rate; 30 per cent of them were prostitutes.

TABLE VIII

*Comparison between histological and colposcopic findings and behavioural characteristics of the 300 prison women over 21 years of age. Physiological epithelium was diagnosed when either a physiological transformation zone was seen colposcopically or where biopsy of an atypical transformation zone revealed only physiological epithelium*

| Behavioural characteristics | Epithelial abnormalities (Histological diagnosis) | | | | Physiological transformation zone (Colposcopic and histological diagnosis) (n = 226) | |
|---|---|---|---|---|---|---|
| | Major (n = 31) | | Minor (n = 43) | | | |
| | No. | Per cent | No. | Per cent | No. | Per cent |
| *Marital status:* | | | | | | |
| Single | 14 | 44 | 11 | 26 | 61 | 27 |
| Married | 12 | 40 | 19 | 45 | 136 | 60 |
| Divorced | 5 | 16 | 13 | 28 | 29 | 13 |
| Nulliparous | 5 | 16 | 11 | 26 | 70 | 31 |
| No contraception | 15 | 48 | 34 | 80 | 136 | 60 |
| First intercourse before age 15 | 9 | 29 | 21 | 48 | 81 | 36 |
| Ten sexual partners or more (includes prostitutes) | 15 | 48 | 16 | 38 | 81 | 36 |

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# "GESTATION SAC" VOLUMES AS DETERMINED BY SONAR IN THE FIRST TRIMESTER OF PREGNANCY

BY

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## Summary

Sonar estimates of "gestation sac" volumes in the first trimester of pregnancy were made from a series of 319 measurements; the volumes estimated included the amniotic fluid, the extraembryonic coelom and the fetus. The technique employed involved the use of parallel section scans taken in series from one end of the gestation sac to the other, followed by planimetric measurements of the sac areas so produced. The potential errors which may be incurred in these measurements are discussed, and it is considered that the technique carries an overall possible error in the order of ±10 per cent. Growth curves produced from the measurements in this series show that the sac increases in volume from a mean of 1 ml. at 6 weeks to a mean of 100 ml. at 13 weeks, initially in an exponential fashion but latterly in a more linear manner. The mean values of the sonar gestation sac fluid volumes (after subtraction of the estimated fetal volume) in the 10 to 13 week range, show good correlation with those amniotic fluid values reported in the literature where direct measurements were made at the time of hysterotomy. As a method of assessing the maturity of a pregnancy this technique is of lesser value than the sonar measurement of fetal crown-rump length because of the relatively wider scatter of results. It has, however, found a useful place in clinical practice in the early sonar diagnosis of blighted ova or anembryonic pregnancies.

OVER the last twenty years amniotic fluid volume estimations in the second trimester of pregnancy have been reported by several authors (Harrison and Malpas, 1953; Monie, 1953; Wagner and Fuchs, 1962; Rhodes, 1966; Abramovich, 1968 and 1970; Gillibrand, 1969; Lind and Hytten, 1970; Smith, 1971), with specimens obtained principally at termination of pregnancy by hysterotomy. This source, however, is not usually applicable to pregnancies in the earlier weeks, as at this stage termination by the vaginal route is the method of choice. The other potential source of information about amniotic sac volumes in the first trimester is that of spontaneously aborted intact gestation sacs, but, because of their pathological nature, the values obtained cannot necessarily be equated with sac volumes of normal pregnancies at equivalent gestations. It followed, therefore, that if reliable information were to be readily obtained about sac volumes at this period of gestation then a different approach would have to be considered. To this end the possibility of using sonar measurements was explored.

In the sonar study of early pregnancy several workers have previously measured diameters of the gestation sac from simple two-dimensional section scans and have published growth curves from the values obtained (Hellman *et al.*, 1969; Jouppila, 1971; Trooswijk, 1972; Kossoff *et al.*, 1974). Such measurements however provide no information on the volume of the sac, particularly as its shape and therefore dimensions may vary greatly depending on the degree of bladder fullness and the position of the uterus in the pelvis. To obviate this problem and to try and determine the actual volume of the gestation sac a technique was developed in which serial parallel section scans of the gestation sac were taken and planimetric measurements made of the sac areas so produced. This paper reports the results of a large series of such measurements in the first trimester of pregnancy.

In the context of this study the term "gestation sac" rather than amniotic sac has been deliberately chosen as, until at least the eleventh week, the fluid filled sac as depicted by sonar comprises both extraembryonic coelom and amniotic cavity (Arey, 1965). From this time onwards the amnion gradually fuses with the chorion, a process which may last until at least the fourteenth week (Scrimgeour, 1974). Any measurement by sonar of the volume of the gestation sac, therefore, includes the combined volumes of the extraembryonic coelom, the amniotic fluid, and of course the fetus.

## METHOD

The sonar apparatus used was the Nuclear Enterprises Diasonograph NE4102. The technique of gestation sac volume measurement is based on the principle that if two flat surfaces are placed in parallel at a predetermined distance apart, and, if the area of each surface is known, the volume of space between the two surfaces can then be estimated. In applying this principle to the gestation sac, serial parallel section scans were made at 0·5 cm. or 1 cm. intervals from one end of the sac to the other thereby subdividing the sac volume into a series of flat truncated cones (Fig. 1a). Intervals of 0·5 cm. were used from the fifth to the tenth week of menstrual age, and 1 cm. thereafter. In those cases in which the gestation sac had a diameter of less than 1 cm., calculation of its volume was made from a measurement of its diameter, as in such cases the sac was almost invariably circular in section. All the scans were displayed on the 0·94 to 1 display scale and were photographed using Polaroid film. The area of gestation sac as shown on each photograph was measured using a Haf Planimeter No. 315, appropriate corrections having been made for the scale factor of 0·94 to 1 and for a further small 3 per cent error in scale registration on the particular storage oscilloscope used.

The method used to estimate the sac volume from the individual gestation sac areas was that of the trapezoidal law for measurement of the area under a curve applied to the three-dimensional situation of the gestation sac. As depicted in a two-dimensional form in Figure 1b, this simply entailed taking the mean area of each pair of parallel areas, multiplying the resulting figure by the distance between the scans and considering the value obtained to be the volume of fluid between the two scans. The volume of the last segment was estimated empirically by halving the area of the last sac section and multiplying the result by distance "D". The volume of the whole gestation sac is then the sum of these individual volumes. In its reduced form the mathematical formula which described this method is simple and practical in its application—:

$$V = \frac{D}{2}\,(A1 + A2 + - - - + An),$$

where V = volume of the sac,
D = distance between the scans and A = area of the sac as depicted on the two-dimensional scan.

### *Sources of potential error*

(*a*) *Beam width.* The beam width effect occurs in ultrasonic scanning where a point reflector is recorded as a short line on the oscilloscope rather than as a single dot. This is due to the fact that the ultrasonic beam has a measurable width and therefore, as the beam sweeps across a point reflector, a returning echo will be produced throughout the time that the point remains within the beam. The lengths of these lines vary with the frequency and power level of the ultrasound being used. The practical result of the beam width effect is that the inner transverse diameter of the gestation sac as depicted on the oscilloscope screen is slightly reduced. In measuring the areas of the gestation sac sections, however, appropriate allowances were made for this artefact, and it was considered that only a small error in the measurements would be incurred because of this problem.

(*b*) *Mathematical considerations.* Since the shape of the gestation sac is irregular and variable it is necessary to use an approximate method of

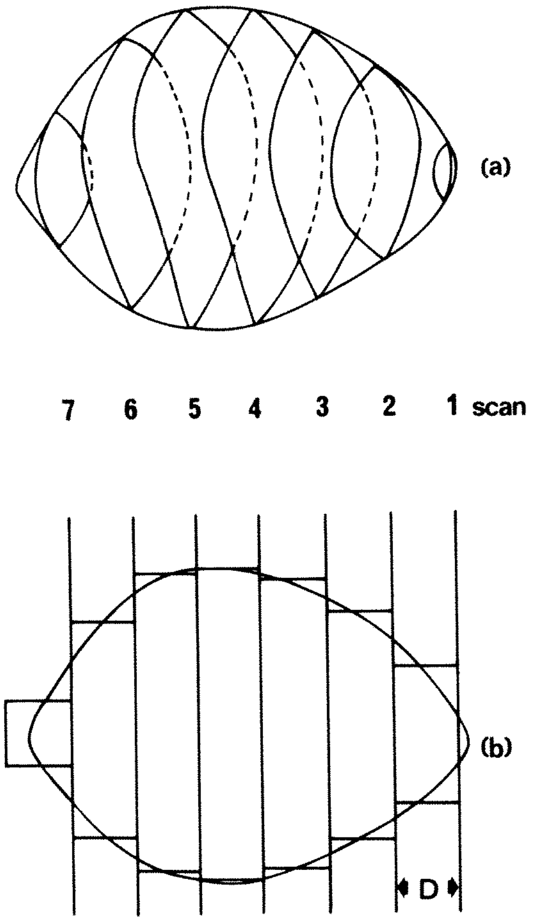

FIG. 1

A diagrammatic representation of the technique employed in the sonar estimation of "gestation sac" volume. The vertical lines correspond to serial parallel section scans taken at either 0·5 cm. or 1 cm. intervals (D). The sac volume is thereby divided into a series of flat truncated cones. The upper part of the figure (*a*) contrives to demonstrate this subdivision in a two-dimensional form. The mathematical method used to estimate the volume of the gestation sac is illustrated in the lower part of the figure (*b*). The mean area of each adjoining pair of areas is calculated and the resulting value multiplied by the distance between them (D), thus giving an estimate for the volume of each "truncated cone". The overall volume of the gestation sac is then taken to be the sum of these volumes.

estimating its volume based on measurement of a series of cross-sectional areas. Simpson's rule would be the method of choice but unfortunately requires that the volume be divided into an even number of slices of equal thickness a basic consideration which would considerably lengthen the scanning time. For this reason Simpson's rule was discarded as being unsuitable. In order to allow the use of an odd or even number of slices a method based on the trapezoidal rule was used as described above. On consideration of the solid geometry of this method it was apparent that there was a potential for a small overestimation in the calculation of the volumes of the individual central "slices" and for an underestimation at the extreme ends and, that because of the variability of the shapes of sacs and the sonar technique involved, a definitive figure for the potential error based on exact mathematical calculations could not be given.

In order to obtain an approximation of the error involved attributable to the calculation method *per se*, three models of actual gestation sacs were constructed and their exact volumes measured. These volumes were then compared with the volumes calculated by the method described above, exact areas of the sections having been measured using squared paper. The percentage difference was found to be 5·3, 4·7 and 1·4 for models of 3·2 ml., 14·3 ml. and 95·6 ml. respectively. In each case the calculated value was the smaller of the two.

This test was obviously on a small sample but it shows the mathematical method used is unlikely to result in a significant error in the estimation of gestation sac volume.

(*c*) *Reproducibility of planimetric measurements*. In assessing the potential error involved in the use of the planimeter the volumes of 50 gestation sacs were calculated from three separate sets of planimetric measurements from each original set of photographs. The group involved in this study included cases from each week of the period under investigation and whose gestation sac volumes ranged from one to 100 ml. The average standard deviation (SD) of the three measurements in this series was ±3·2 per cent.

(*d*) *Reproducibility of gestation sac measurements*. In order to determine the reproducibility of the technique *per se* rather than that of the planimetric measurements as in (*c*) above, three separate sets of sonar scans were performed on each of 24 patients, and 5 sets in a further 5 patients. Each set of scans was made with an interval of at least five minutes and often 10 to 15 minutes, to allow bladder filling, or emptying, thereby changing the shape of the gestation sac. In the first group the average SD was ±5·9 per cent, while in the second and smaller group the equivalent value was ±5·4 per cent.

(*e*) *Correlation between sonar and direct measurements*. Such correlations should, at least in theory, reflect the additive effect of the potential errors of the technique described above in the practical situation. Unfortunately, in most cases of abortion the sac wall is partially or completely devitalized making clear definition of its outline by sonar more difficult, thereby incurring a greater potential error in mensuration than in normal pregnancies. A further problem is the difficulty in obtaining suitable cases because of the relative infrequency of having performed a sonar estimation of the gestation sac volume and obtaining post abortion an intact sac for direct fluid measurement all within a short period of time, for example, 48 hours. Despite these problems it was considered that such a correlative study would be of value. Table I shows the values obtained in nine patients who aborted spontaneously within two days of the sonar scan. The estimations of fetal volume used in this Table were derived from the figures given by Streeter (1921) for weight against crown-rump length, and assumed a specific gravity for the fetus of 1·0 (Harrison and Malpas, 1953). The maximum error was to be found in patient number 4 where the difference was 4·0 ml., or 16 per cent. The average difference was 6·4 per cent. The closest correlations were to be found in two patients (1 and 5) in which the tissues of the sac were noted to be fresh. In general there was a tendency for the sonar estimation of volume to be higher than the direct *post abortum* measurements. No judgement, however, can be made as to whether this overestimation is due to measurement error, bearing in mind the poor nature of the tissues, or to loss of fluid from the sac during the abortion process. Notwithstanding, these factors and the small numbers involved in the close correlation between the sonar and direct measurements as shown in this study would suggest that the technique, in more normal pregnancies, should be a reasonably accurate one. This study would also suggest that the potential errors, (*a*) to (*e*), in the practical situation, tend to cancel each other rather than be additive.

In the light of these considerations, it is felt that an estimate of an overall potential error in the order of ±10 per cent should include all but the very worst cases.

## RESULTS

A total of 319 examinations was performed on 125 patients, all of whom were sure of the dates of their last menstrual periods and had normal, regular, menstrual cycles. In all patients beyond six and a half weeks of menstrual age, confirmation of a living pregnancy was made by demonstration of fetal heart movements (Robin-

TABLE I

*Comparison of sonar estimates of gestation sac fluid volumes and the volumes measured directly from the intact aborted sac. In each case spontaneous abortion took place within 48 hours of the sonar scan. F = Fresh tissue; P.N. = Partially Necrotic Tissue*

| Patient No. | Fetal crown-rump length (mm.) | Sonar gestation sac volume (ml.) | Estimated fetal volume (ml.) | Sonar fluid volume (ml.) i.e. gestation sac volume-fetal volume | Direct fluid volume (ml.) | Direct volume-sonar volume (ml.) | Percentage difference | Tissue |
|---|---|---|---|---|---|---|---|---|
| 1 | 8 | 1·0 | 0·2 | 0·8 | 0·8 | 0 | 0 | F |
| 2 | – | 4·8 | – | 4·8 | 4·3 | −0·5 | 11·6 | P.N. |
| 3 | 18 | 16·6 | 0·8 | 15·8 | 15·2 | −0·6 | 3·9 | P.N. |
| 4 | 14 | 21·5 | 0·5 | 21·0 | 25 | 4·0 | 16·0 | P.N. |
| 5 | 36 | 21·8 | 3·2 | 18·6 | 18·5 | −0·1 | 0·5 | F |
| 6 | 17 | 27·9 | 0·7 | 27·2 | 28 | 0·8 | 2·9 | P.N. |
| 7 | 16 | 29·5 | 0·6 | 28·9 | 28 | −0·9 | 3·2 | P.N. |
| 8 | 19 | 31·5 | 1·0 | 30·5 | 28 | −2·5 | 8·9 | P.N. |
| 9 | 22 | 37·6 | 1·1 | 36·5 | 33 | −3·5 | 10·6 | P.N. |

son, 1972) and confirmation of maturity by the sonar measurement of fetal "crown-rump length" (Robinson, 1973). Only those patients in whom the gestation sac outlines were well defined were included in the study, as its principal object was to determine normal values for gestation sac volumes with the minimum of measurement error. This qualification, however, excluded less than 5 per cent of the possible patients from the series. Figure 2 shows a section of a gestation sac and fetus at just under 11 weeks gestation.

In order to reduce the scatter of results the volume estimations were analyzed in groups of three days rather than seven days. On examination of the data in each of these groups it was found that the results exhibited a log-normal distribution and therefore the mean and 2 SD values were calculated from the logarithms of the results. The values obtained are shown in Table II and the same data is graphically displayed in Figure 3 in its smoothed form together with a curve of the mean gestation sac fluid volumes after subtraction of the estimated fetal volumes, which were derived from the data of Streeter (1921) as described earlier. The crown-rump length values used in these calculations were those obtained by Robinson (1973).

TABLE II

*Mean and 2 SD values of "gestation sac" volumes in intervals of three days*

| Duration of amenorrhoea (days) | Number of examinations | Mean "gestation sac" volume (ml.) | Upper second standard deviation | Lower second standard deviation |
|---|---|---|---|---|
| 35–37 | 7 | 0·2 | 1·1 | 0·04 |
| 38–40 | 6 | 0·5 | 1·1 | 0·2 |
| 41–43 | 7 | 1·0 | 1·7 | 0·5 |
| 44–46 | 15 | 1·8 | 6·5 | 0·5 |
| 47–49 | 15 | 2·3 | 6·6 | 0·8 |
| 50–52 | 15 | 4·5 | 13·2 | 1·5 |
| 53–55 | 22 | 5·9 | 18·6 | 1·9 |
| 56–58 | 21 | 11·2 | 33·4 | 3·8 |
| 59–61 | 22 | 12·0 | 29·5 | 4·9 |
| 62–64 | 24 | 17·8 | 38·0 | 8·3 |
| 65–67 | 20 | 22·4 | 51·3 | 9·8 |
| 68–70 | 30 | 29·5 | 51·3 | 17·0 |
| 71–73 | 26 | 38·0 | 81·3 | 17·4 |
| 74–76 | 20 | 44·7 | 75·9 | 25·7 |
| 77–79 | 18 | 53·7 | 102·3 | 27·5 |
| 80–82 | 17 | 61·7 | 102·3 | 36·3 |
| 83–85 | 19 | 74·1 | 114·8 | 46·8 |
| 86–88 | 6 | 77·6 | 112·2 | 55·0 |
| 89·91 | 5 | 109·6 | 173·8 | 67·6 |
| 92–94 | 4 | 104·7 | 141·3 | 75·9 |

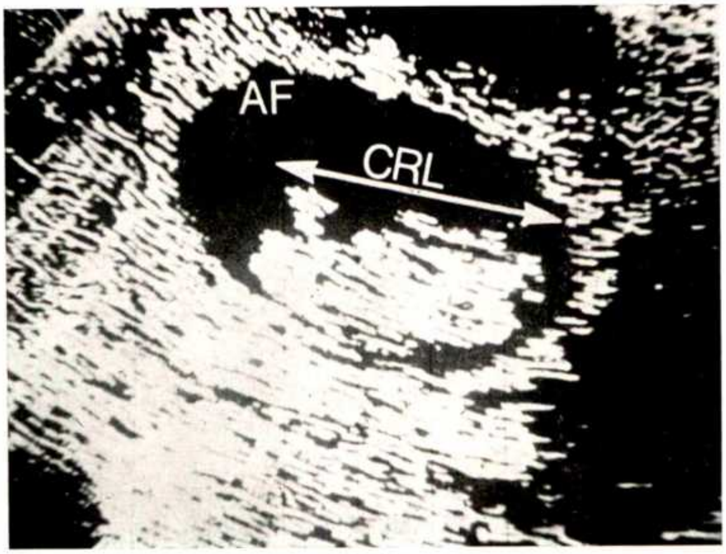

FIG. 2

A pregnancy at 10 weeks and 6 days of menstrual age in which the fetus has a "crown-rump length" (CRL) of 41 mm. and a "gestation sac" volume of 45 ml. AF = amniotic fluid. Scale 0·94 : 1.

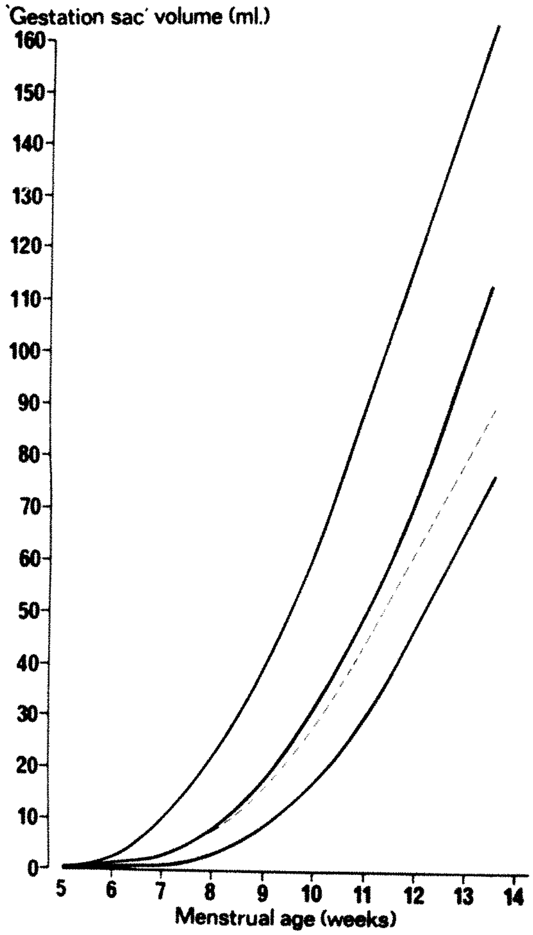

FIG. 3
The smoothed mean and 2 SD values (continuous lines) of 319 gestation sac volume estimations from 5 to 14 weeks of amenorrhoea. The broken line represents the mean gestation sac volume after subtraction of the estimated fetal volume.

From the graph it can be seen that the mean gestation sac volume increases exponentially from a value of 1 ml. at 6 weeks to 31 ml. at 10 weeks, and then, in a more linear fashion, to 100 ml. at 13 weeks, with corresponding values of 1 ml., 28 ml., and 80 ml. for the fluid volume alone. This rapid increase in volume of the gestation sac is paralleled by a scatter of results which increase with advancing gestation.

## DISCUSSION

Hellman *et al.* (1969), Jouppila (1971), Troostwijk (1972) and Kossoff *et al.* (1974), have all demonstrated ultrasonically the rapid growth of the "gestation sac" in the first eleven weeks of pregnancy by means of growth curves of gestation sac diameters. These observations attempted to quantify the subjective descriptions reported by Donald (1967 and 1969). While these observations and measurements infer an increase in volume of the gestation sac with advancing pregnancy they are unable to demonstrate such an increase in absolute terms. Furthermore, depending on the degree of bladder filling, the shape of the gestation sac may vary from that of a sphere to almost pancake dimensions, thereby making comparisons between sac sizes an inaccurate and potentially misleading procedure. The technique described in this investigation, however, within the limits of the experimental errors described, does provide absolute values for the volume of the gestation sac and is independent of its shape, thus allowing direct comparisons to be made between the sac volumes of different pregnancies, and in the same pregnancy at different gestational ages.

The mean values of gestation sac volumes as determined in this study, after correction for estimated fetal volume, are close to the values obtained by direct measurement in the 10 to 13 weeks range (Table III).

TABLE III

*Comparison of sonar "gestation sac" fluid volumes and direct values of amniotic fluid volumes from specimens obtained at hysterotomy as reported in the literature (Wagner and Fuchs, 1962; Rhodes, 1966; Abramovich, 1968; Gillibrand, 1969).*

| Gestational age | Sonar volumes | | Direct volumes | |
|---|---|---|---|---|
| | Number of cases | Mean (ml.) | Number of cases | Mean (ml.) |
| 10+ | 50 | 37 | 9 | 30 |
| 11+ | 46 | 53 | 10 | 50 |
| 12+ | 19 | 70 | 14 | 76 |
| 13+ | 5 | 90 | 13 | 95 |

The direct values used in this Table are the means of the values reported by Wagner and Fuchs (1962), Rhodes (1966), Abramovich (1968), and Gillibrand (1969) for amniotic fluid volumes and, for ease of comparison, values midway between each completed week of menstrual age were used for both the sonar and the direct measurements. While these values are not absolutely comparable, because of the inclusion of the extraembryonic coelom in the sonar measurements, it is probable that this cavity is quite small by this stage in pregnancy. Arey (1965) states that the amniotic cavity fills the chorionic sac at the end of the second month (10 weeks of menstrual age), and that loose fusion of the amnion and chorion then occurs. There is evidence, however, from those workers who have undertaken fetoscopy in human pregnancies that secure fusion may not be complete even at the fourteenth week of pregnancy (Scrimgeour, 1974). If it is accepted that the extraembryonic coelom does not contribute a significant proportion of sonar gestation sac volume after the tenth week then the close correlation of the values as shown in Table III offers indirect evidence for the validity of the sonar values earlier in pregnancy where no direct comparative figures are available.

It is of interest in the context of this study that at no time was any structure observed within the gestation sac which could confidently be recognized as the amniotic membrane itself. The probable explanation for this is that the acoustic impedance of the very thin amnion is insufficient to reflect a discernible echo at the sonar power levels normally employed and with the apparatus currently in use.

The scatter of results found in this series increases, as would be expected, with advancing gestation, and at 13 weeks the 2 SD limits extend from 65 ml. to 145 ml. As the gestation sac forms the major part of the contents of the gravid uterus beyond 8 or 9 weeks, an appreciation of this potential range in sac volume is of considerable importance when an assessment of gestational age is being made on the basis of a clinical examination. Furthermore, because of this relatively wide scatter of results, the use of the technique itself as a means of estimating maturity is of lesser value than the sonar measurement of fetal crown-rump length (Robinson, 1973). The maturity range in the former method at 12 weeks, for example, is ± nine days, while in the latter it is only ± four days. But volume estimations have in the author's experience, been found to be of considerable value in the five to seven week range before the fetus is visible, and in the early diagnosis of anembryonic pregnancies or blighted ova.

In all the descriptions so far published on the sonar appearances of the developing gestation sac, reference has been made to an apparent breaking up or dissolution of the sac wall at about the eleventh week of pregnancy (Donald, 1967; Donald and Abdulla, 1967; Hellman *et al.*, 1969; Kohorn *et al.*, 1969; Jouppila, 1971; Troostwijk, 1972). Contrary to these reports, however, it was often found in the course of this investigation that it was possible to delineate completely the margins of the gestation sac up to and beyond the thirteenth and fourteenth weeks of pregnancy.

This discrepancy raises the obvious question of why it has been possible in this study to outline the sac wall beyond the hitherto accepted limit of 11 weeks. Before attempting to answer this question it is pertinent to review the theories which have been put forward to explain this apparent dissolution of the sac. Donald (1967), who first described this feature attributed it to the differentiation of the chorion frondosum and the chorion laeve, while Hellman and his co-workers (1969) presumed that it represented the fusion of the decidua capsularis with the decidua parietalis, thereby eliminating an interface for reflection of the ultrasonic waves. Finally, Troostwijk (1972) gave as his explanation the complete filling of the uterine cavity by the developing sac. Of these theories, the first would seem to have the best embryological basis, and, from a sonar standpoint, the loss of the trophoblastic shell from the greater part of the surface of the sac would, in effect, result in the elimination of a band of ideal sound-reflecting surfaces thereby making it more difficult to obtain good echoes from the sac wall. If this is indeed the explanation then there would be no reason to suppose that the fusion of the decidua capsularis (or chorion laeve) with the decidua parietalis occurs any earlier than the normally accepted time of 14 to 16 weeks (Arey, 1965; Hamilton and Mossman, 1972).

In the initial stages of this study it was found that by using slightly higher power levels of the emitted ultrasound it was possible to compensate for this loss of sound-reflecting surfaces, thereby allowing clearer delineation of the inner surface of the sac wall, but this resulted in an unbalanced picture, with "overexposure" of other structures such as the uterine wall and bladder outline. To resolve this problem two steps were taken. Firstly the scanning technique was changed from the commonly employed compound linear scan to one with little or no compounding, and secondly, the life-size display scale was used instead of a one-third scale, this allowing the operator to ignore any remaining "overexposure" of surrounding structures, by their simple exclusion from the screen.

A second factor which is probably of some importance in the ability to outline the sac is, that with the fixed time varied gain or receiver attentuation system common in the older ultrasonic machines, the echoes from some parts of the now fairly large sac may be over-suppressed causing an artefactual "break" in the sac wall. The apparatus used in this study had a manually-controlled and therefore variable receiver attenuation system, and its use helped to obviate the occurrence of any such artefacts.

It is therefore concluded that the ability to delineate the outline of the gestation sac in this study was due to the use of higher sonar power levels, the change in scanning technique, and the greater versatility of the sonar apparatus used.

## ACKNOWLEDGEMENTS

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# ULTRASONIC "B" SCANNING AS A PREGNANCY TEST AFTER LESS THAN SIX WEEKS AMENORRHOEA

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## Summary

Sixty patients with less than six weeks amenorrhoea were examined by ultrasonic "B" scanning for the detection of pregnancy. The results were compared with those obtained by histological examination of the aspirate after the patient had undergone menstrual regulation by vacuum aspiration of the uterine contents. The overall accuracy was 79 per cent with 15 per cent false positives. The accuracy after 39 days amenorrhoea was 95 per cent. The technique was compared with an immunological pregnancy test.

THE diagnosis of early pregnancy by clinical examination and immunological urine testing can often be misleading. Because of the biological variation of pregnancy testing, accuracy is doubtful and false negative results may occur, particularly where there has been less than five weeks amenorrhoea. With the introduction of diagnostic ultrasound, it is possible to identify the gestation sac within the uterus as early as the fifth week of pregnancy and to provide objective information for the diagnosis of pregnancy (Donald, 1967 and 1969).

The ultrasonic examination of early pregnancy is helpful in assessing its continuation in cases of threatened abortion and in determining fetal maturity (Robinson, 1972 and 1973). This report describes the use of ultrasound in the diagnosis of early pregnancy (less than 42 days amenorrhoea) and correlates the results and accuracy with the immunological urine testing in those patients undergoing very early termination of pregnancy (menstrual regulation by vacuum aspiration of uterine contents).

## METHOD

Patients undergoing vacuum aspiration of early pregnancy were scanned prior to the procedure for the presence of a gestation sac within the uterus. The study was restricted to women who had reported their menstrual period delayed between one and fourteen days. Most of the subjects had an initial immunological pregnancy test (Pregnosticon Dri-Dot test, Organon Inc.) performed. This urinary pregnancy test is a latex agglutination slide test designed to detect human chorionic gonadotrophin (HCG) levels of 1500 IU per litre. The screening pregnancy test was interpreted as "positive" when no agglutination was observed, "negative" if definite agglutination occurred and "indefinite" if agglutination could not be detected with certainty.

The apparatus used for scanning was the Nuclear Enterprises Diasonograph 4102. Using the full bladder technique as described by Donald (1963), a midline longitudinal "B" scan was first made and the uterus located. If any difficulty was encountered a series of transverse scans was made to clarify the position of the uterus since it was often deviated by the full bladder. The plane of scan was then adjusted to contain the long axis of the uterus. Having located the correct plane of scan, the picture scale was expanded from one-fifth of full size to full size and the whole of the uterus searched for the presence of a gestation ring. If a ring was visualized it was photographed. If not, a scan of the largest section of the uterus was made and a photographic record kept.

Three operators took part in this trial, each experienced in obstetric scanning. A short learning period was allowed before the trial began. After each scan the operator assigned a score to the patient of 1, 2, 3 or 4, according to the diagnosis of not pregnant, probably not pregnant, probably pregnant, and definitely pregnant. The diagnosis was formulated on a number of considerations. The most important of these were:

(*a*) the presence or absence of a gestation ring;
(*b*) the dimensions of the uterus;
(*c*) the presence of organized echoes within the uterine cavity;
(*d*) only disorganized echoes or no echoes from within the uterine cavity.

Patients in whom a ring could be seen (Fig. 1) were assigned the score of 4 regardless of the other factors. Patients with an enlarged uterus and with some organization of the echoes from within the uterus (Fig. 2) were assigned a score of 3. Patients with only a slightly enlarged uterus and with echoes from the uterine cavity suggesting only the presence of menses (Fig. 3) were assigned a score of 2, and patients with little enlargement of the uterus or no apparent echoes from within the cavity (Fig. 4) were assigned a score of 1.

The area of the uterus in the median sagittal plane was estimated as the product of the maximum length and width of the uterus as measured from the scan. A normalized area was

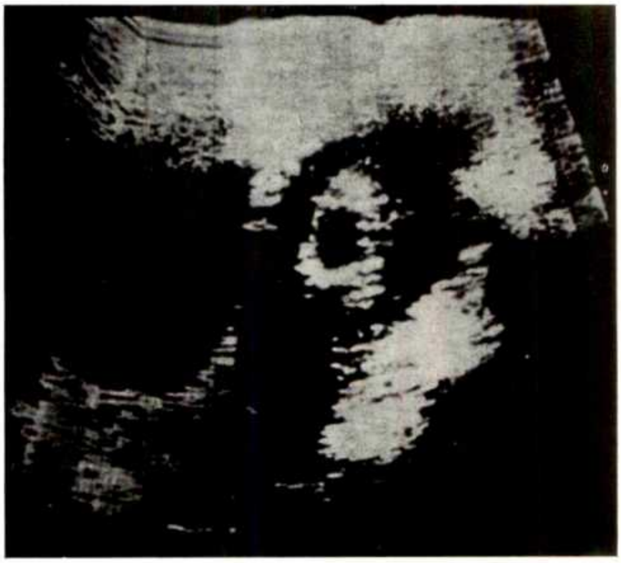

FIG. 1

An example of a gestation ring visualized in the cavity of the uterus. This scan clearly merits a score of 4.

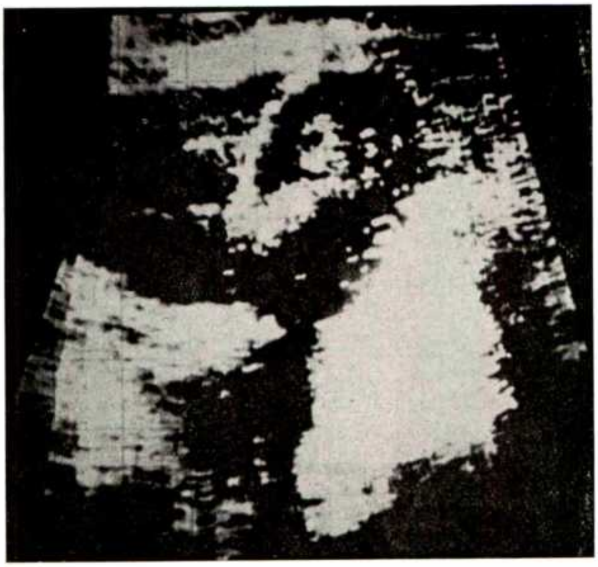

FIG. 2
Organized echoes from within the uterine cavity. The decision criteria suggested led to a score of 3 being assigned to scans of this type.

calculated by taking the ratio of the area to the days of amenorrhoea.

Vacuum aspiration was performed without general anaesthesia on an outpatient basis. The volume of the uterine contents was recorded and subjected to histological examination. The diagnosis of pregnancy by pathological examination of the aspirate required the presence of products of conception. The histological evaluation and volume of the aspirate were correlated with the scan score, the uterine area, the normalized area and days of amenorrhoea.

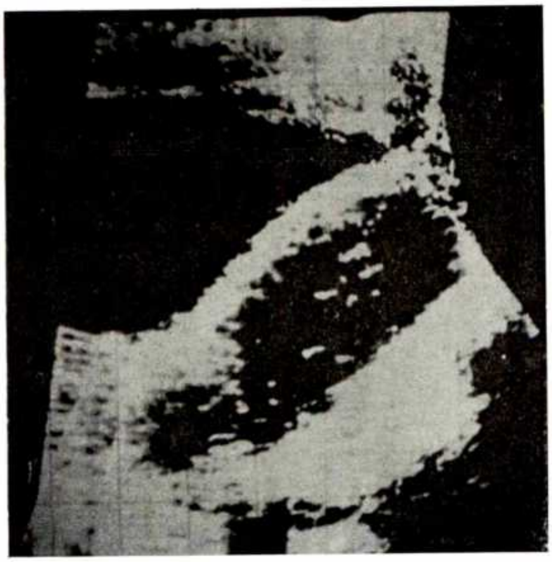

FIG. 3
Disorganized echoes from within the uterine cavity on a scan assigned a score of 2.

## RESULTS

Sixty patients were included in the trial. Of these, 4 were eliminated because of equivocal histology. Of the remainder, the histology showed 34 to be pregnant, 13 to be non-pregnant and a further 9 were considered not to be pregnant since menstruation occurred subsequent to the scan but prior to aspiration. Evaluation on the scan score (Table I) showed 40 pregnant and 16 not pregnant. There were nine false positives, two of which were given a score of 4, and three false negatives, one of which was given a score of 1. Thus 22 per cent of those diagnosed as positive were in fact not pregnant, and 19 per cent of those diagnosed as not pregnant were actually pregnant. These results correspond to an overall accuracy of 79 per cent, with 15 per cent false positive and 5 per cent false negative.

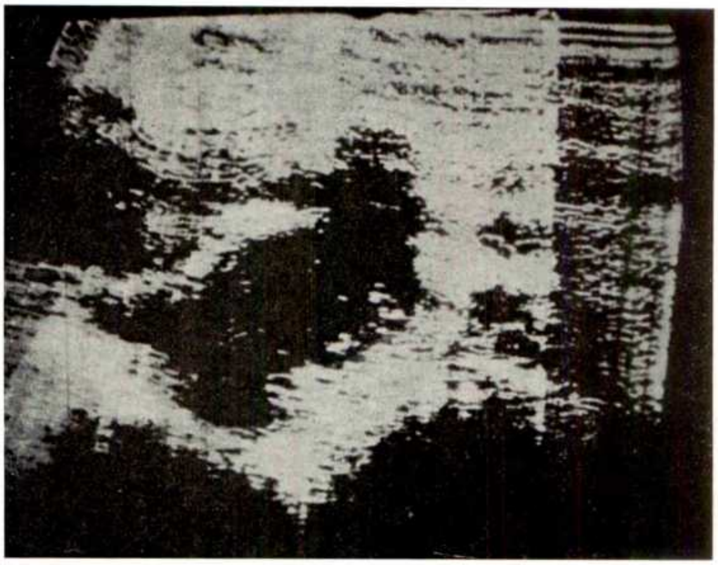

FIG. 4

A scan typical of those scoring 1, showing no obvious echoes from within the uterine outline.

This result is significantly better than random allocation of diagnosis based on a knowledge of the likelihood of pregnancy ($\chi^2=14\cdot16$ with one degree of freedom, $p=<0\cdot001$).

The accuracy in the 11 patients who had less than five weeks amenorrhoea was 82 per cent and 76 per cent in the 45 patients who had more than five, but less than six weeks amenorrhoea. There were 22 patients with more than 39 days amenorrhoea. In this group the only diagnostic error was a pregnant patient assigned a score of 2.

The score correlated strongly with the volume of the aspirate ($\chi^2=35\cdot7$ with nine degrees of freedom, $p=<0\cdot001$), as did the uterine size ($\chi^2=32$ with 12 degrees of freedom, $p=<0\cdot001$).

An immunological pregnancy test was performed in 44 patients in this series. The result was equivocal in three patients and incorrect in three patients, all three errors being false negatives.

The receiver operator characteristic (Fig. 6; see Appendix) was interpolated as a smooth curve from the four points generated by the evaluation score. Since there is a continuum of scores which could have been assigned to the population of scans performed, this over-simplification is justified for the purpose of rank ordering. However, it cannot be used with confidence with iso-information curves as proposed by Metz *et al.* (1973). The characteristics

TABLE I

*Summary of results*

| Score | 1 | | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|---|---|
| Days amenorrhoea | 28–42 | 39 | 28–42 | 39 | 28–42 | 39 | 28–42 | 39 |
| Pregnant according to histology | 1 | 0 | 2 | 1 | 14 | 8 | 17 | 9 |
| Not pregnant | 3 | 1 | 10 | 3 | 7 | 0 | 2 | 0 |

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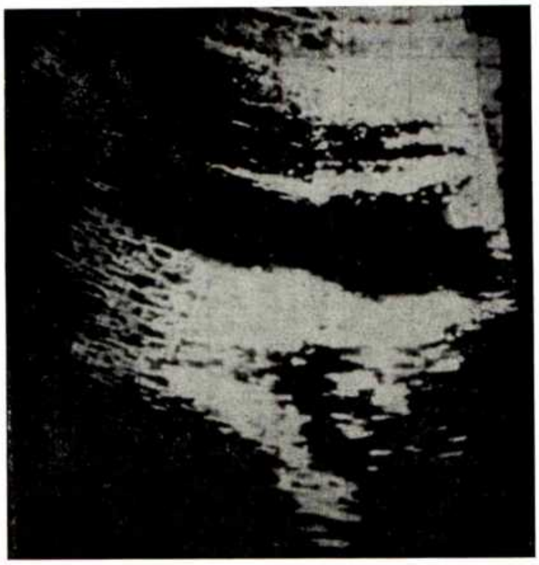

FIG. 5

A scan assigned a score of 4 because of the partial ring of echoes at the bottom right of the picture. Histology did not confirm our diagnosis of pregnancy.

show that the evaluation score was better than a test based on uterine size or normalized uterine size alone. Patients were scored as far as possible without conscious reference to the period of amenorrhoea. The characteristic of this period is included as if it were a diagnostic test to show the improbability that it was a dominant factor in the diagnosis. The one point generated by the immunological test is shown for completeness. The correlation between the histology and the volume of aspirate is clear from the characteristic.

### DISCUSSION

Those cases with a clear ring or a completely empty uterus were easy to classify. The difficulty occurred in establishing which of the scans with disorganized echoes represented early pregnancies and which related to the disintegration of endometrium prior to menstruation. The uterine size offered a useful indication, but often the operator had to rely on a rather unsatisfactory subjective impression, incapable of precise definition.

Retrospective analysis of the two scans given a 4 rating, but which were histologically negative, showed, in one case, a circular extrauterine structure, possibly a loop of bowel which was taken to be intrauterine, and, in the other case, what must have been artifactual echoes from the menses fortuitously giving the impression of a ring (Fig. 5). The scan given a score of 1, which was subsequently related to pregnancy, involved misdiagnosis of the position of the uterus, which was retroverted.

"B" scanning is at least as successful diagnostically as immunological pregnancy testing where there has been less than six weeks amenorrhoea. It is safe and rapid, but more likely to give a false positive result since menses may be diagnosed as products of conception. In addition, this type of error is reduced as the experience of the operator increases. False negative results are common with routine pregnancy tests. Tulchinsky and Hobel (1973) concluded that during the first seven weeks of pregnancy the diagnosis of intrauterine pregnancy cannot be made with certainty by the determination of steroid hormones. Recently, Kessel (1974) reported that an immunological (Dri-Dot) pregnancy test, in those patients undergoing early termination of pregnancy, had a false negative rate as high as 29 per cent, when correlated with the histology of the evacuated uterine contents. Certainly a more accurate estimation of HCG is by radioreceptorassay, which is sensitive and highly specific (Landesman and Saxena, 1974). Detection of the hormone as early as day one following the first period missed has been reported. This is a highly sophisticated technique which is unsuitable for routine pregnancy testing. The use of ultrasound offers a useful application in the diagnosis of early pregnancy.

### ACKNOWLEDGEMENTS

We record our gratitude to Dr. J. Stringer for her unstinting efforts to encourage her patients to volunteer for this examination and for carrying out the immunological pregnancy tests. Our thanks are due to Professor D. V. I. Fairweather for suggesting the trial and for his continued interest and encouragement. We acknowledge with thanks the advice and assistance in the interpretation of scans given by Miss G. C. L. Lachelin, Senior Registrar in the Department of Obstetrics and Gynaecology. We are grateful to Mrs. Heather Preece for her efficient secretarial assistance.

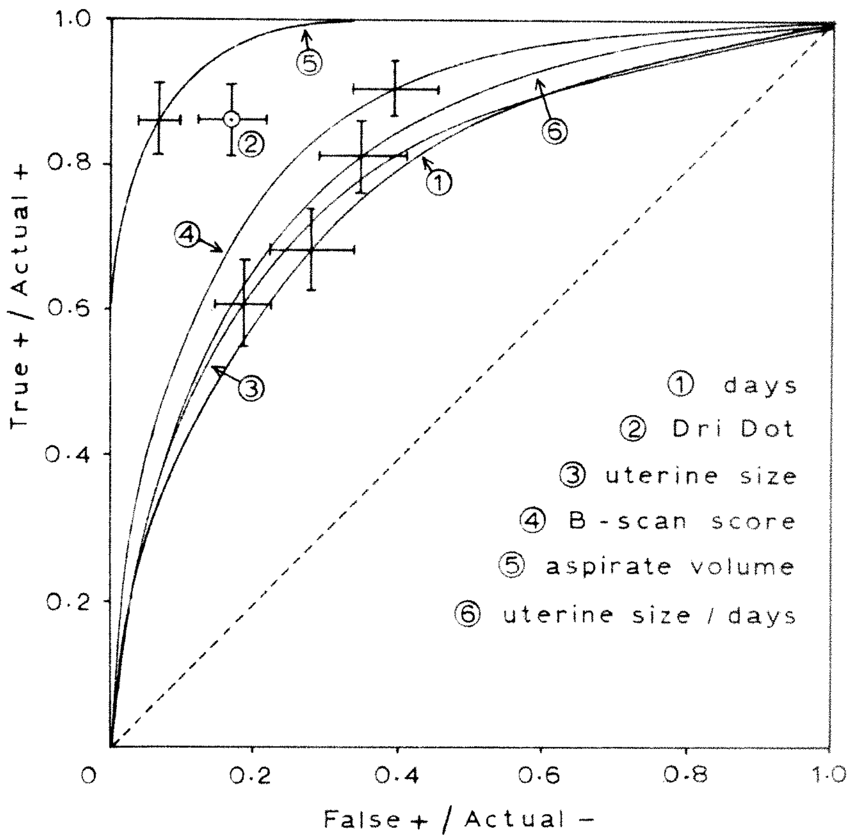

FIG. 6

The receiver operator characteristics of the variables measured. Predicted standard deviation bars are shown to indicate the probable variation in the position of each characteristic.

## APPENDIX

### RECEIVER OPERATOR CHARACTERISTIC

The receiver operator characteristic (Swets, 1964) provides a graphical means of determining, at least in rank order, the relative efficiency of a number of diagnostic techniques. In approximate terms, the closer a curve or characteristic is to the top left hand corner of the graph the more efficient is the diagnostic technique. The receiver operator characteristic is applicable to the situation where the diagnosis has a yes/no result, in this case pregnant/not pregnant. The curve is generated as a plot of the true positive/actual positive ratio with the false positive/actual negative ratio as the diagnostic decision criterion is altered. As an illustration, consider the uterine size as a test of pregnancy. A decision level that all uterine areas above 12 cm.$^2$ relate to pregnancy generates one point on the characteristic, since a certain number of gravid uteri will be smaller than this, thus giving rise to a true positive number which is less than the actual positive number. When the decision level is changed, say to 15 cm.$^2$, another point is generated. A cavalier diagnostician who says "If the uterus has any size at all then it is pregnant" would generate the point at the top right hand corner of the graph, whereas a timid diagnostician who took the view that the uterus was never pregnant would generate the point at the bottom left. If uterine size had no relationship to pregnancy then the results, as the decision level was changed, would be randomly distributed and the characteristic would lie along the positive diagonal. The further the characteristic is from this diagonal the stronger the ability of the test to separate the yes/no groups. The relative usefulness of the tests may be evaluated at the level of false positive or false negative which the user is prepared to tolerate.

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# THE DIAGNOSIS OF FETAL HYDRONEPHROSIS, MEGAURETER AND URETHRAL OBSTRUCTION BY ULTRASONIC ECHOGRAPHY

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## Summary

Two cases of fetal urethral obstruction, one with megaureter and obvious hydronephrosis, were diagnosed by two-dimensional ultrasonic echography. With good quality echograms it should be possible to make the diagnosis early in pregnancy.

IN establishing antenatal care, Ballantine (1901) hoped to detect and so prevent congenital malformations. With the development of X-rays, fetal bony abnormalities were soon diagnosed but soft tissue malformations proved much more difficult to detect. It is only recently that ultrasound has made it possible to examine the fetal urinary tract. The normal fetal bladder was distinguished within the fetal abdomen six years ago (Robinson *et al.*, 1968) and a case of fetal polycystic kidney has been reported (Garrett *et al.*, 1970). The frequency of fetal micturition and the fetal urinary output have been measured by detailed study over many hours and, in a series of 20 patients, the maximum bladder volume before micturition has been recorded as 40 ml. (Campbell *et al.*, 1973). With the introduction of grey scale to two-dimensional ultrasonic echography (Kossoff, 1972; Kossoff and Garrett, 1972) it has been possible to distinguish fine detail in the parenchyma of solid tissues and so be partly independent of the demonstration of outlines for anatomical identification. The higher quality grey scale echograms give more useful information and are a pointer to the direction in which echoscope design must go if we are to achieve Ballantine's original goal.

Two cases of fetal urethral obstruction, one partial and the other functionally complete, are described in which the diagnosis was made by ultrasonic echography.

## CASE REPORTS

*Patient 1.* A patient, aged 32, in her fourth pregnancy, was unsure of the date of her last menstrual period. To assist with the management, her obstetrician requested ultrasonic echography to measure fetal size. The pregnancy had otherwise been normal.

The echograms taken with the National Acoustic Laboratories' Mark II Water Delay Abdominal Echoscope showed a single fetus, vertex, presentation, lying in the right occipito-anterior position. Both the fetal head and fetal trunk corresponded in size with the usual measurements at 34 weeks (Garrett and Robinson, 1971). The fetal bladder was of normal size and apparently contained 20 ml. of urine. The kidneys showed bilateral hydronephrosis with particularly marked distension of the renal pelvis and flattening of the renal tissue posteriorly. With high-grade grey-scale echograms the renal tissue was seen to be one shade towards grey compared with the echographic blackness of the urine in the renal pelvis establishing the dark grey area as solid tissue and therefore kidney. On the fetal right a 5 mm, wide ureteric orifice to the bladder could be seen (Fig. 1). The volume of amniotic fluid was normal. Congenital urethral stenosis or obstruction was diagnosed and when the patient was informed she told of herself having had repeated urethral dilatations up to the age of twelve years and of her mother having but one kidney and having died of uraemia.

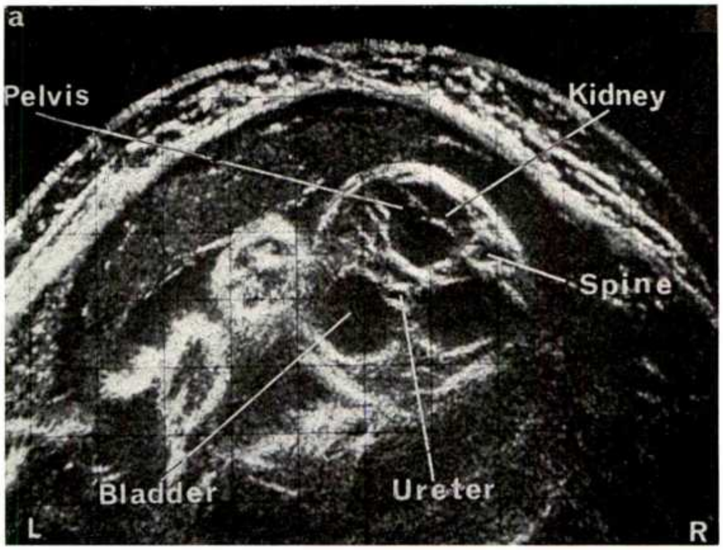

FIG. 1

*Patient 1.* Transverse section echogram 1 cm. above the umbilicus. The plane of section is sloping. It passes through the fetal abdomen showing a normal-sized fetal bladder anteriorly. The kidneys are compressed posteriorly by the dilated pelves of the ureters. There was a normal volume of amniotic fluid. Comparison of the size of the head and the fetal abdomen showed no increase in fetal size.

Three weeks later the examination was repeated. Both the fetal head and trunk then corresponded in size with the usual measurements at 37 weeks. There was no distension of the fetal abdomen but the bladder was larger than usual and was thought to contain 45 ml. of urine. The renal pelves and ureters were virtually indistinguishable and the right ureteric orifice (Fig. 2) now measured 15 mm. across. Repeated examination of the fetal bladder over four hours showed its volume to vary between 31 and 50 ml. and at no time to be empty. It was not certain whether the lower bladder volumes were achieved by passing urine *per urethram* or by reflux to the ureters.

Labour was induced and an apparently healthy female baby weighing 3·17 kg. was delivered. The umbilical cord contained three vessels. The kidneys were not clinically enlarged but the bladder could be palpated and gentle pressure over it expressed a little urine. The baby voided urine spontaneously soon afterwards.

At seven days, the baby was examined with the N.A.L. Contact Echoscope and 85 ml. of residual urine was demonstrated in the bladder 15 minutes after micturition. The baby progressed quite normally gaining weight and showing no apparent difficulty with micturition. At nine weeks an intravenous pyelogram (Fig. 3) confirmed the original ultrasonic diagnosis. Bilateral hydronephrosis with ballooning of the renal pelvis and flattening of the renal parenchyma was found exactly as had been demon-

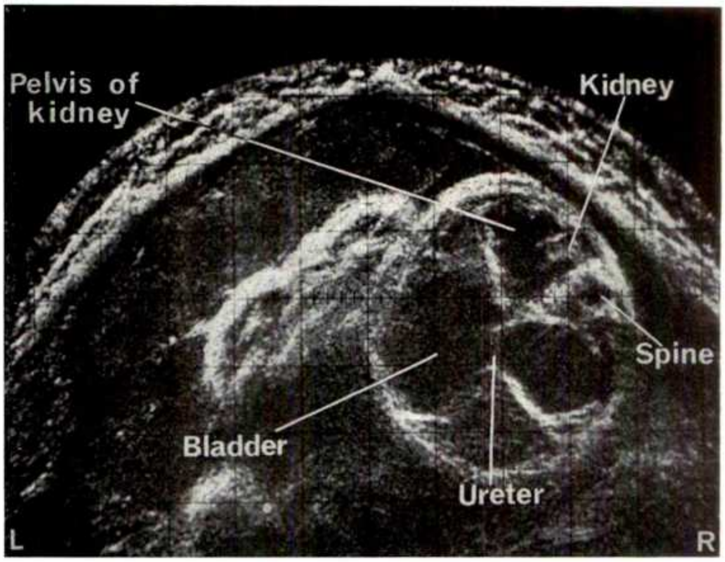

FIG. 2

*Patient 1.* Transverse section echogram taken three weeks after Figure 1. The fetal bladder is now bigger than normal and the ureters are grossly dilated. The fetal right ureteric orifice is 15 mm. in diameter.

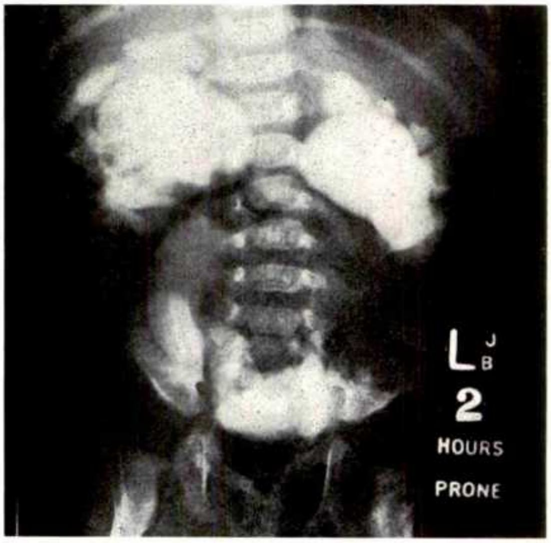

FIG. 3

*Patient 2.* Intravenous pyelogram confirming the ultrasonic findings of bilateral hydronephrosis.

strated in the echograms. The megaureter on the right side showed gross dilatation of the ureteric orifice. The bladder, though larger than normal, was not grossly dilated.

*Patient 2.* The patient was a healthy woman aged 23 years, who had had one normal previous pregnancy. At the 32nd week of her second pregnancy the obstetrician referred her for ultrasonic echographic examination with a request to measure fetal size. The echograms showed a normal fetal head corresponding in size to the period of amenorrhoea. The fetal chest was of normal size but the abdomen was grossly distended by a monolocular cystic structure containing 3000 ml. of liquid (Fig. 4). The umbilical vein which was 6 mm. in diameter, could be seen and appeared to be stretched over the top of the cystic structure showing that its origin was in the lower abdomen and was either fetal bladder or, perhaps a mesenteric cyst (Fig. 5). On either side of the fetal spine compressed tissue was noted which appeared to contain the kidneys. The volume of amniotic fluid was within normal limits.

Eight days later the examination was repeated. The findings were confirmed. The cystic structure now appeared to contain 3500 ml. of liquid and the diagnosis of gross distension of the bladder due to congenital urethral obstruction was made. Needle aspiration of the bladder removed two litres of urine and repeat ultrasonic examination showed a small quantity of free fluid in the fetal peritoneal cavity around the partly collapsed bladder (Fig. 6). The patient came into labour spontaneously and was delivered the following day at the 34th week of pregnancy of a stillborn male fetus weighing 2·86 kg. It was necessary to aspirate a further 200 ml. of urine from the fetal bladder to relieve obstruction in the second stage. This urine contained 35 mmol./l. sodium, 3 mmol./l. potassium, 33 mmol./l. chloride, less than 50 mg./l. protein (but no albumin), 0·5 mmol./l. glucose, and the osmolality was 91 mmol./kg.

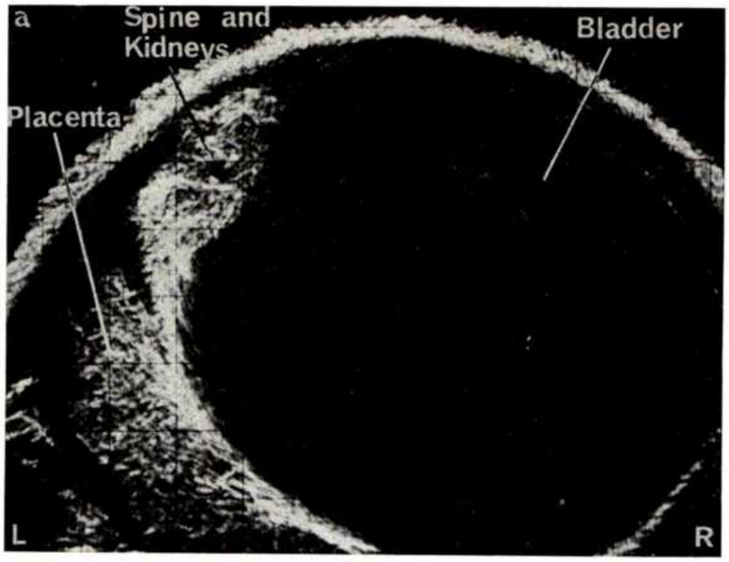

FIG. 4

*Patient 2*. Transverse section echograph 7 cm. above the umbilicus showing the grossly dilated fetal bladder, 20 cm. in diameter. The lower abdomen was much larger than the upper abdomen (Fig. 5) and the fetal head.

At necropsy, the distended smooth walled bladder still contained 970 ml. of clear urine. There were small bilateral hydroureters and hydronephrosis and the apex of the bladder was at the umbilicus. The left umbilical artery was normal but the right umbilical artery was represented by a single fibrous strand. The umbilical cord contained two vessels. The undescended testes were on the surface of the bladder about 10 cm. above the inguinal rings. Approximately 0·5 cm. of urethra could be demonstrated from above and the lower penile urethra was patent for 1 cm. In the intervening 2 cm. it was not possible to demonstrate a lumen macroscopically but sections showed a narrow lumen which was double for a short distance. It is thought that this arrangement led to a functional, if not an absolute, anatomical block. The anus was

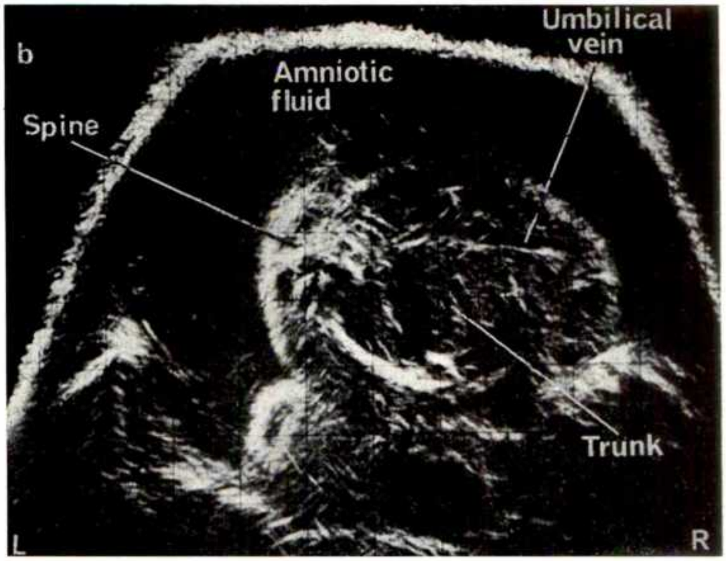

FIG. 5

*Patient 2*. Transverse section echogram 11 cm. below the plane in Figure 4. The umbilical vein is stretched across the top of the dilated bladder. The volume of amniotic fluid appears to be normal.

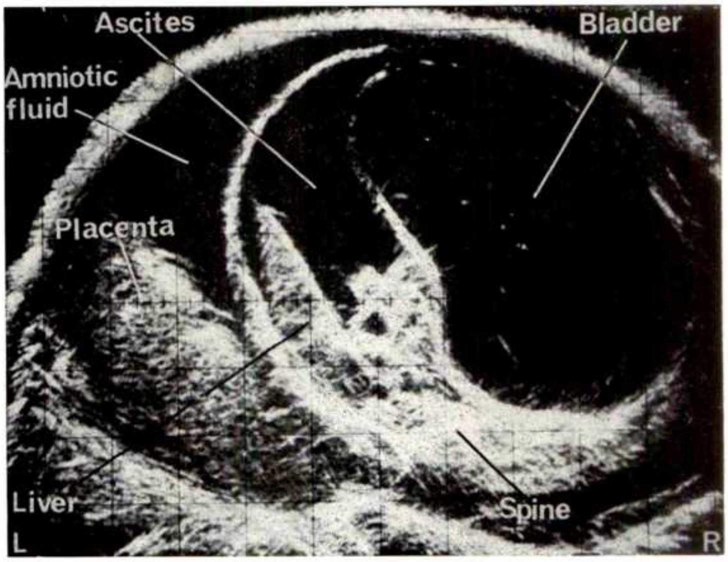

FIG. 6

*Patient 2*. Transverse section echogram through lower fetal abdomen after needle aspiration of 2 litres of fetal urine. The fetal ascites is now clearly seen.

patent. The liver was flattened against the diaphragm and the stomach and bowel were compressed posteriorly.

## CONCLUSION

The two cases of fetal urethral obstruction described show that the diagnosis is not difficult given good quality echograms. In one case the request was prompted by the unusual abdominal palpation but in the other the finding was mere accident. In the patient with gross distension of the fetal bladder the delivery was difficult but had the diagnosis been unknown this difficulty would have been compounded by the less well directed manipulations by the obstetrician with consequent increased risks to the mother. In the other patient there was a family history of congenital abnormalities of the urinary tract and, from the examples given, it now seems worthwhile to submit all patients with such a history to ultrasonic examination for the early diagnosis of affected fetuses. With high resolution equipment, it should be possible to diagnose a gross case in early pregnancy allowing selective abortion if appropriate.

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# THE AETIOLOGY, CHARACTERISTICS AND DIAGNOSTIC RELEVANCE OF LATE DECELERATION PATTERNS IN ROUTINE OBSTETRIC PRACTICE

BY

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## Summary

A retrospective study is described in which all the late deceleration patterns seen in one obstetric unit over the course of 34 months have been reviewed with regard to their apparent aetiology, their characteristics and associated abnormal fetal heart rate patterns and the incidence of fetal distress. The results show that, regardless of the aetiology and the characteristics, the incidence of fetal distress (defined as a pH of less than 7·25 or an Apgar score of less than 7 at one minute) lies between 40 and 50 per cent. The implications of this degree of diagnostic inaccuracy are discussed and the importance of fetal blood sampling as the final arbiter in the diagnosis is emphasized. During the course of the study a significant relationship was demonstrated between the use of epidural analgesia and the incidence of late deceleration patterns. This finding is discussed.

THE term late deceleration has been applied to transient decelerations of fetal heart rate which commence during a uterine contraction, some 20 to 30 seconds after the onset of that contraction, and persist for 30 to 60 seconds after the end of the contraction (Hon, 1959; Hon and Quilligan, 1967). Clinical observation has shown the frequent association between this type of deceleration and fetal acidosis (Kubli *et al.*, 1969; Beard *et al.*, 1971) and as a result a late deceleration pattern is considered almost diagnostic of fetal distress, especially in the presence of other fetal heart rate abnormalities (Beard *et al.*, 1971). Unfortunately, the association is not invariable and the use of cardiotocography alone, without the aid of fetal blood sampling, is likely to result in some unnecessary operative deliveries.

This retrospective study was carried out to assess the true relevance of late deceleration patterns in clinical practice and to determine whether, by studying more closely the apparent aetiology and the characteristics of each late deceleration pattern and its associated abnormal fetal heart rate patterns, the diagnosis of fetal distress by cardiotocography alone might be made more reliable. In the course of the study an apparently strong relationship between the use of epidural analgesia and the appearance of late deceleration patterns was found and examined.

## METHODS

Each routine intrapartum continuous fetal heart trace obtained from the labour ward between July 1971 and May 1974 was analysed by the author. Especial attention was paid to late deceleration patterns which were defined as follows.

A late deceleration pattern was said to have developed if five or more late decelerations occurred consecutively. A late deceleration was defined as any transient deceleration of heart rate exceeding 10 beats per minute in depth, the nadir of which occurred more than 30 seconds after the peak of the contraction.

Labour ward notes and information recorded on the traces were used to determine the sequence of events leading up to and following each late deceleration pattern. An attempt was made to determine the aetiology of the late deceleration pattern in each case. The state of each fetus was assessed using the results of fetal blood sampling and the state of each newborn baby was determined from the one and five-minute Apgar scores and the relevant notes in the labour ward record.

During the 34-month period studied there were 5549 deliveries of which 946 were monitored either because of clinical fetal distress or as indicated by "at risk" factors. Late deceleration patterns occurred in 79 cases (8·4 per cent). It was necessary to exclude two cases from the study in view of insufficient information regarding the labour.

## RESULTS

### *The relevance of late deceleration patterns in terms of fetal distress*

The one-minute Apgar score was less than 7 in 36 cases (47 per cent) and the five-minute Apgar score was less than 8 in 11 of these. Relevant fetal blood samples were taken in 42 patients and the resulting pH was less than 7·25 in 17 (41 per cent). The overall incidence of fetal compromise in terms of either fetal scalp blood pH or the one-minute Apgar score was 47 per cent.

### *The apparent aetiology of late deceleration patterns*

Table I shows the factors associated with the appearance of late decelerations. The pattern developed within two to fifteen minutes of administration of epidural anaesthetic and/or the adoption of the supine or lithotomy position in 55 patients (71 per cent). There were no obvious aetiological factors in 15 patients (19 per cent). In three patients there was obvious uterine hypertonia, in one the fetus was severely affected by rhesus isoimmunization and in the remaining three patients prematurity was considered as a possible aetiological factor. Nuchal encirclement by the umbilical cord was present in 21 patients. The incidence of fetal compromise lay between 40 and 50 per cent in each of the aetiological groups except where there was no proven factor in which group the incidence was not significantly different at 53 per cent.

TABLE I

*The factors associated with the appearance of late deceleration*

| Factor | Total number | Patients with fetal distress | |
|---|---|---|---|
| | | No. | Per cent |
| Epidural alone | 12 | 5 | 42 |
| Supine position or lithotomy | 24 | 12 | 50 |
| Epidural and supine position or lithotomy | 19 | 8 | 42 |
| Uterine hypertonia | 3 | 1 | – |
| Prematurity | 3 | 1 | – |
| Rhesus isoimmunisation | 1 | 1 | – |
| No obvious cause | 15 | 8 | 53 |
| Associated factor: | | | |
| Nuchal encirclement by cord | 21 | 10 | 48 |

### *The relationship between epidural analgesia, late deceleration patterns and fetal compromise*

This was investigated using figures obtained in the last twelve months of the study following marked changes in labour ward policy regarding fetal monitoring, epidural analgesia, and documentation.

During the last twelve months of the study 252 epidural anaesthetics were given and 124 were monitored. Late deceleration patterns were observed in 25·8 per cent of these compared with 5·2 per cent of the 344 monitored labours which were managed without epidural analgesia in the same period. This difference is statistically significant ($p = < 0\cdot001$ $\chi^2$ test; 1 degree of freedom).

Despite the significantly higher incidence of late deceleration patterns in the epidural group the incidence of fetal compromise in terms of fetal scalp blood pH and one-minute Apgar score was almost identical in the epidural and non-epidural groups (28·0 and 28·5 per cent respectively).

### *The characteristics of the late deceleration patterns*

The pattern exceeded 60 minutes duration in 35 patients (45·5 per cent) and resolved within one hour in 32 patients (41·5 per cent). In the remaining 10 patients delivery occurred within the 60 minute period. Fifty-seven per cent of the persistent patterns were associated with fetal compromise compared with 34 per cent in the short-lived group. This difference fails to reach statistical significance ($p=<0{\cdot}1$). There was no significant difference between the mean depth of late decelerations in the compromised and uncompromised groups, the values were $40\pm13$ (SE 3·4) and $37\pm14$ (SE 2·9) respectively.

### *Associated abnormal fetal heart rate patterns*

Table II shows the incidence of other fetal heart rate abnormalities occurring in association with late decelerations in the compromised and uncompromised groups. Tachycardia (more than 160 beats per minute for ten minutes or more) and obvious narrowing of the baseline variability to less than 5 beats per minute were present either individually or together in 20 compromised fetuses (59 per cent) and 14 uncompromised fetuses (41 per cent). This difference is not statistically significant ($p=<0{\cdot}1$). Bradycardia was seen in six patients and in only one case was the fetus acidotic.

## DISCUSSION

Published reports on the use of electronic fetal heart rate monitoring have shown the necessity of using the method as a screening procedure and not as a final arbiter in the diagnosis of fetal distress (Beard *et al.*, 1971; Shenker, 1973). Despite such reports the cardiotocograph is still used alone, without resort to fetal blood sampling, in some obstetric units. This study was set up to determine whether such use of the cardiotocograph is justified and to investigate possible methods of improving the diagnostic accuracy of the fetal heart rate trace. The results show that only half of the fetuses demonstrating late deceleration patterns show any other signs of compromise and that, even by looking at the apparent clinical cause, the size of the decelerations, the duration of the pattern and associated abnormalities of heart rate, there was no way of distinguishing the compromised from the uncompromised fetuses without further investigation in the form of fetal blood sampling.

TABLE II

*The incidence of associated abnormal fetal heart rate patterns*

| Abnormal pattern | Total number | Incidence of fetal distress | |
|---|---|---|---|
| | | No. | Per cent |
| Silent baseline variability | 21 | 13 | (62) |
| Tachycardia | 24 | 15 | (62) |
| Silent baseline variability and tachycardia | 11 | 8 | (73) |
| Silent baseline variability and/or tachycardia | 34 | 20 | (59) |
| Bradycardia | 7 | 1 | (14) |
| Cord occlusion pattern | 11 | 7 | (64) |

It may be argued that the criteria used in this study for a late deceleration pattern were not strictly correct according to classical usage. Eleven of the late deceleration patterns were suggestive of cord compression by virtue of the shape and depth of the decelerations and these would therefore have been labelled as "variable" according to the accepted classification (Hon and Quilligan, 1968). This was felt to be unimportant as both variable and late decelerations are associated with fetal distress and it would therefore seem logical to include late, variable decelerations in the clearly defined late deceleration group rather than in the vague variable deceleration group. Seven of the eleven fetuses involved were distressed.

The significant association between epidural analgesia and late deceleration patterns was an interesting finding in the light of previous prospective trials designed to investigate the effect of epidural analgesia on fetal heart rate (Noble *et al.*, 1971; Schifrin, 1972). Noble *et al.* (1971) found an 18 per cent incidence of maternal hypotension related to epidural analgesia but there was no significant difference between the respective incidences of late decelerations in the epidural group and a non-epidural group. Schifrin (1972) found that late decelerations occurred in 72 per cent of cases in which similarly defined hypotension occurred and there was an incidence of 23 per cent even in the absence of measurable hypotension. This latter figure agrees closely with the incidence of late decelerations associated with epidural analgesia in this present study. It is not usual, in this unit, to experience large falls in maternal brachial blood pressure associated with epidural analgesia but it is possible, as Schifrin (1972) suggests, that this measurement does not necessarily reflect the uterine artery pressure upon which intervillous perfusion depends. There is evidence to suggest that during normotension the uterine artery pressure, as reflected by pressure in the femoral artery, is equal to the brachial blood pressure but that during hypotension, whatever the degree of severity, the fall is 1·4 times greater in the uterine artery than it is in the brachial artery. This effect has been attributed to aortic compression (Bieniarz *et al.*, 1966). The discrepancy between the fetal heart rate findings of Noble *et al.* (1971) and those of Schifrin (1972) and this present study requires examination. It is probably related to epidural technique and it is possible that speedier turning of the patient from the supine to a lateral position following an epidural top-up might have resulted in a lower incidence of fetal heart rate abnormalities. The three studies are agreed that the use of epidural analgesia makes no difference in terms of perinatal outcome.

The supine and lithotomy positions appeared to be responsible for the appearance of late decelerations in the absence of epidural analgesia in 15·6 per cent of the patients in this study. The supine hypotension syndrome is thought to occur in only 2 per cent of pregnant patients (Holmes, 1960), but as its appearance depends on the inability of the collateral venous circulation to compensate for compression of the inferior vena cava (Kerr *et al.*, 1964) it might reasonably be expected that a spectrum exists between no supine hypotension and the complete syndrome. As described above it is possible for a small fall in brachial blood pressure to be accompanied by a greater fall in uterine artery pressure.

The information accrued in this study suggests that a large proportion of late deceleration patterns occur secondarily to transient lowering of the uterine artery blood pressure caused either by compression of the abdominal aorta or by a fall in cardiac output secondary to compression of the inferior vena cava or by a combination of the two. Whether or not acidosis develops depends on the state of the fetus at the onset of labour and presumably on the duration of the aetiological insult although it was not possible to prove that in this study. Animal experiments have shown that late decelerations are a reflection purely of fetal hypoxia and that correction of the hypoxia may bring about resolution of the heart rate despite the continued presence of acidosis (James *et al.*, 1972; Myers *et al.*, 1973). It is apparent that, in view of the inconstant relationship between late deceleration patterns and fetal scalp blood pH, serial estimations of pH are necessary in order to determine the true state of fetal well-being.

In conclusion, a review of all the late deceleration patterns occurring in one obstetric unit in a 34-month period has shown the necessity of carrying out fetal blood sampling in addition to cardiotocography and that there is no justification for the use of cardiotocography alone. A significant relationship has been observed between the use of epidural analgesia, the adoption of the supine or lithotomy positions and the appearance of late decelerations.

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# CONTINUOUS PETHIDINE/DIAZEPAM INFUSION DURING LABOUR AND ITS EFFECTS ON THE NEWBORN

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## Summary

This paper presents a study comparing the effects on the mother and baby of a continuous intravenous infusion of pethidine and diazepam and of intramuscular pethidine and promazine. It is concluded that the administration of intravenous pethidine and diazepam is safe in labour provided that diazepam has not been given during the previous week.

IN an effort to formulate a method of analgesia which provided good pain relief, had an adequate tranquillizing effect, had no ill-effects on the fetus or the newborn and gave some degree of amnesia following delivery, we compared the effect of intramuscular pethidine and promazine with the effect of intravenous pethidine and diazepam.

## METHODS

### *Mothers*

A total of 150 patients was studied. In 75 cases an infusion of 250 mg. of pethidine and 40 mg. of diazepam dissolved in 500 ml. of 5 per cent dextrose/saline was used. The drip rate was controlled by a Decca 460 automated infusion unit. Initially the infusion was allowed to flow freely until satisfactory relief was obtained and thereafter the drip rate was controlled by the patient's response to uterine contractions. The infusion was started when the patient asked for pain relief, no notice being taken of the stage of labour which had been reached, and it was continued until the second stage.

In the other 75 patients (of which 49, or 65 per cent, were primigravidae) analgesia was provided by intermittent injections of 100 mg. of pethidine, together with 50 mg. of promazine. The first injection was given when it was considered that the patient needed pain relief and the injections were repeated as required. None of the patients had obstetric abnormalities and the methods of analgesia were applied alternately.

In order to obtain some degree of uniformity it was decided to induce all patients at term, provided the cervix was favourable, and in each case intravenous oxytocin was given from the time of induction. However, in the intravenous pethidine/diazepam group, oxytocin was not given from the onset in 7 patients, so these were excluded from the study, leaving only 68 patients in this group (of which 38, or 56 per cent, were primigravidae). The oxytocin was administered according to the following regime: 2 units of oxytocin in 500 ml. of 5 per cent dextrose/saline was administered, starting at 10 drops per minute, increasing the dose by 10 drops per minute at half-hourly intervals until a maximum of 60 drops per minute was reached. If labour had not yet become established, the dosage of oxytocin was increased to 5 units per 500 ml. of 5 per cent dextrose/saline using the same dosage schedule.

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In an attempt to assess the degree of analgesia on an objective basis, two groups of observers were used. A detailed questionnaire was completed by the midwife looking after the patient in labour and the degree of the analgesia obtained was based on her observations. Twenty-four hours later, two trained nurses who were unaware of the type of analgesia that had been used during labour, discussed the overall impression of labour with the patient. The degrees of analgesia and amnesia were assessed as fair, good or very good. The paediatrician assessed the condition of the baby at the time of delivery using the Apgar score.

### *Infants*

Twenty infants were studied after their mothers had received pethidine and diazepam by continuous infusion up to the second stage of labour. They were compared with 30 control infants whose mothers had received other methods of analgesia. Particular attention was paid to the temperature within the first three hours of life, neuromuscular state, tone, reflexes, feeding and respiratory effort. In the infants born to mothers given intravenous pethidine and diazepam, samples of maternal and cord blood were taken at delivery and capillary blood samples were taken from the infants when other routine investigations were being carried out in the early neonatal period. Pooled urine samples were obtained during the first two days of life. All samples were stored frozen until ready for analysis. Diazepam and its metabolites were analyzed by electron capture gas chromatography (Baird *et al.*, 1973). Urine samples were incubated with $\beta$-glucoronidase before extraction and chromatographic analysis. Blood levels of diazepam and its active metabolite desmethyldiazepam were estimated up to and including the tenth day of life in some cases.

## RESULTS

### *Mothers*

The mean total duration of labour was 610 minutes for the intramuscular pethidine/promazine group and 590 minutes for the intravenous pethidine/diazepam group. The respective mean total doses of pethidine for the two groups were 130 mg. and 157 mg.

Table I shows the analgesia produced by the two regimes. This assessment was made by the midwife at the time of labour. In each case this impression was confirmed by a questionnaire completed 24 hours later.

The data were analyzed by means of the $\chi^2$ test. The first test was to determine whether the response of the primigravidae differed significantly from the multigravidae in each group. In each case there was no evidence of a significant difference. The two methods of treatment were then compared. Comparing all patients receiving pethidine/diazepam with those receiving pethidine/promazine, $\chi^2(2) = 14\cdot6$ ($P<0\cdot001$). There is, therefore, strong evidence that intravenous

TABLE I

*Comparative analgesia achieved by each group on a percentage basis*

| Degree of analgesia | Intravenous pethidine/diazepam | | | | Intramuscular pethidine/promazine | | | |
|---|---|---|---|---|---|---|---|---|
| | Number of primiparae | Number of multiparae | Total | | Number of primiparae | Number of multiparae | Total | |
| | | | No. | Per cent | | | No. | Per cent |
| Fair | 6 | 1 | 7 | 10·3 | 17 | 8 | 25 | 33·3 |
| Good | 15 | 18 | 33 | 48·5 | 25 | 11 | 36 | 48·0 |
| Very good | 17 | 11 | 28 | 41·2 | 7 | 7 | 14 | 18·7 |

*Apgar scores at one and fiv*

| | | 2 | | 3 | | 4 | | |
|---|---|---|---|---|---|---|---|---|
| | | No. | Per cent | No. | Per cent | No. | Per cent | No |
| Assessment at one minute | Intramuscular pethidine/promazine | 1 | 1·5 | – | – | 6 | 8·8 | 8 |
| | Intravenous pethidine/diazepam | – | – | 5 | 6·7 | 4 | 5·3 | 7 |
| Assessment at five minutes | Intramuscular pethidine/promazine | – | – | – | – | – | – | – |
| | Intravenous pethidine/diazepam | – | – | – | – | – | – | – |

pethidine/diazepam gives more effective analgesia than pethidine/promazine given by intramuscular injections.

There was some difference in the response of primigravidae and multigravidae. There was a significantly greater degree of amnesia in the primigravidae who were given pethidine/diazepam, but no difference in the degree of amnesia was found in multiparae.

*Infants*

The distribution of Apgar scores in the two groups is shown in Table II and no significant difference can be detected.

Table III gives clinical information about the 20 infants who were studied more closely for ten days after delivery to a mother given intravenous pethidine and diazepam during labour. These infants were compared with 30 control infants whose mothers had been given analgesia in the form of pethidine and promazine. In each group the temperature within the first three hours of life was carefully recorded. The incidence of initial low body temperature did not differ from the control group and in the only infant (No. 12) showing lethargy, the mother had been given large doses of pethidine/diazepam and also papaveretum and delivery was under general anaesthesia. Three infants required intubation and ventilation. In two of these cases this followed obstetrical complications and administration of only very small doses of diazepam to the mother. The third infant (No. 10) became apnoeic after an initially good Apgar score. The mother had received 24 mg. of diazepam but the infant showed a high cord serum level (0·0181 μg./ml.). Mean blood levels of diazepam and desmethyldiazepam and their ratio during the first ten days of life are shown in Table IV. The ratio of diazepam to desmethyldiazepam decreases with time, suggesting that the newborn is capable of metabolizing diazepam by N-demethylation. There is an unexpected peak in the mean blood concentrations of diazepam and desmethyldiazepam on the seventh day of neonatal life. This was a general tendency and probably indicates mobilization from the tissue depots.

Table V shows the mean urine levels of diazepam and its metabolites for the 20 babies studied closely. The major urinary metabolite was desmethyldiazepam with relatively low levels of the hydroxylated compounds oxazepam and 3-hydroxydiazepam.

## DISCUSSION

The blood level results complement those of a previous study (Cree *et al.*, 1973) and confirm some of the findings of the earlier work. As in the previous work, appreciable individual variations in the blood level curves were found. Cord levels of both diazepam and desmethyldiazepam were higher than those in the maternal circulation, confirming the results of earlier work (de Silva *et al.*, 1964; Scher *et al.*, 1972; Erkkola *et al.*, 1973). After day 1, the diazepam concentration decreased while that of the metabolite persisted in significant concentrations until day 10. It is of interest that the cord serum ratio of diazepam to its metabolite was higher than *the two groups of patients*

| | Apgar score | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 6 | | 7 | | 8 | | 9 | | 10 | |
| nt | No. | Per cent | No. | Per cent | No. | Per cent | No. | Per cent | No. | Per cent |
| | 8 | 11·8 | 14 | 20·6 | 19 | 27·9 | 12 | 17·6 | – | – |
| | 14 | 18·7 | 17 | 22·7 | 16 | 21·3 | 12 | 16·0 | – | – |
| | – | – | 6 | 8·8 | 9 | 13·2 | 30 | 44·1 | 23 | 33·8 |
| | – | – | 1 | 1·3 | 13 | 17·3 | 44 | 58·7 | 17 | 22·7 |

TABLE III

*Clinical details and maternal dosage of intravenous pethidine/diazepam in cases studied in the neonatal survey*

| Mother | | | | Infant | | | |
|---|---|---|---|---|---|---|---|
| Patient number | Method of delivery | Total pethidine dose (mg.) | Total diazepam dose (mg.) | Gestational age (weeks) | Birth weight (kg.) | Apgar score at one minute | Temperature within 3 hours of birth (°C.) |
| 1 | Forceps | 175 | 17 | 40 | 2·75 | 9 | 36·1 |
| 2 | Normal | 100 | 20 | 40 | 3·2 | 6 | 36·3 |
| 3 | Forceps (persistent occipito-posterior) | 200 | 40 | 40 | 3·27 | 7 | 36·8 |
| 4 | Normal | 200 | 40 | 40 | 3·03 | 9 | 35·2 |
| 5 | Normal | 180 | 32 | 40 | 3·31 | 9 | 36·2 |
| 6 | Forceps | 200 | 40 | 40 | 3·75 | 9 | 36·2 |
| 7 | Caesarean section | 200 | 40 | 41 | 3·17 | 6 | 36·2 |
| 8 | Normal | 100 | 20 | 41 | 3·71 | 7 | 36·5 |
| 9 | Normal (precipitate) | 80 | 16 | 40 | 3·47 | 2 | 35·2 |
| 10 | Normal | 120 | 24 | 41 | 3·47 | 8 | 36·3 |
| 11 | Normal | 50 | 4 | 41 | 3·84 | 6 | 36·3 |
| 12 | Caesarean section | 300 | 60 | 40 | 3·7 | 8 | 36·2 |
| 13 | Normal | 120 | 24 | 38 | 3·53 | 8 | 35·4 |
| 14 | Normal | 200 | 40 | 40 | 4·25 | 9 | 36·3 |
| 15 | Normal | 120 | 24 | 40 | 3·55 | 9 | 36·3 |
| 16 | Normal | 200 | 40 | 39 | 2·99 | 9 | 37·1 |
| 17 | Normal | 200 | 40 | 37 | 3·39 | 6 | 36·2 |
| 18 | Normal | 150 | 10 | 42 | 3·24 | 9 | 37·0 |
| 19 | Forceps (High cavity rotation) | 600 | 20 | 39 | 3·41 | 6 | 36·2 |
| 20 | Breech extraction | 400 | 30 | 41 | 2·92 | 2 | 37·1 |

Complications:

Patient 9: Precipitate labour. Pneumothorax.
Patient 10. Apgar score fell at 6 minutes. Intermittent positive pressure oxygen given.
Patient 12: Mother given papaveretum during labour. Baby lethargic.
Patient 20: Breech extraction. Intermittent positive pressure oxygen given.

TABLE IV

*Diazepam and desmethyldiazepam blood levels in neonates (μg./ml.)*

| Sample | Diazepam level (μg./ml.) | | Desmethyldiazepam level (μg./ml.) | | Ratio of mean diazepam/mean desmethyldiazepam levels |
|---|---|---|---|---|---|
| | Mean | SE | Mean | SE | |
| Maternal | 0·042 | 0·004 | 0·032 | 0·009 | 1·312 |
| Cord | 0·066 | 0·012 | 0·037 | 0·010 | 1·784 |
| 1 day | 0·063 | 0·020 | 0·045 | 0·015 | 1·400 |
| 2 days | 0·046 | 0·011 | 0·091 | 0·035 | 0·505 |
| 3 days | 0·037 | 0·006 | 0·069 | 0·021 | 0·536 |
| 4 days | 0·032 | 0·023 | 0·073 | 0·009 | 0·438 |
| 5 days | 0·015 | 0·005 | 0·068 | 0·020 | 0·221 |
| 6 days | 0·014 | 0·008 | 0·099 | 0·068 | 0·141 |
| 7 days | 0·036 | 0·020 | 0·174 | 0·057 | 0·207 |
| 8 days | 0·006 | 0·001 | 0·060 | 0·012 | 0·100 |
| 10 days | 0·005 | 0·003 | 0·065 | 0·028 | 0·077 |

TABLE V

*Levels of diazepam and its metabolites in urine collected from the newborn during the first 24 hours of life*

| | Mean | SD |
|---|---|---|
| Diazepam (μg./ml.) | ·0055 | ±·0015 |
| Desmethyldiazepam (μg./ml.) | ·0445 | ±·0135 |
| Hydroxydiazepam (μg./ml.) | ·0085 | ± 0045 |
| Oxazepam (μg./ml.) | ·0089 | ±·0024 |

the maternal figure. This may indicate that the newborn is able to metabolize diazepam but eliminates the drug more slowly than the adult (Sereni *et al.*, 1972).

The low levels of the hydroxylated metabolites of diazepam in urine from the newborn reflect the lack of hydroxylating and/or conjugating enzyme activity. Our results resemble those of Morselli *et al.* (1973), although they did not detect hydroxylated metabolites until day 18.

In the present study, adverse effects were not observed in the period when blood levels of diazepam and desmethyldiazepam were still appreciable, and it would appear that the term infant is capable of tolerating moderate doses of the drug. It is unlikely that diazepam was responsible for the condition of those infants requiring intubation.

Diazepam and its metabolites have long half-lives, and maternal and fetal blood and tissue levels will rise during prolonged administration of the drug. Because of this, we feel that it is only safe to advocate the use of diazepam during labour in those patients who have not received diazepam in the previous week. Secondly, diazepam and desmethyldiazepam are present in the breast milk of mothers taking the drug. Attention has been drawn to this by Patrick *et al.* (1972), and Erkkola and Kanto (1972), and the administration of diazepam to the nursing mother may, therefore, be contra-indicated.

The intravenous infusion of drugs is potentially dangerous and so it is essential that the patient should be under close and constant supervision by trained staff throughout the labour. If the drugs are infused too rapidly, unconsciousness may result with danger to both mother and fetus.

## CONCLUSION

Pethidine and diazepam given by continuous infusion was found to provide a safe and satisfactory form of analgesia during labour. When the drugs were given in the manner prescribed, no resultant ill-effects were observed. The blood and urine analyses of diazepam and its metabolites indicate that the term infant metabolises and eliminates diazepam more slowly than the adult.

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# THE RENAL CLEARANCE OF OESTRIOL IN LATE HUMAN PREGNANCY

BY

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## Summary

The renal clearance of unconjugated oestriol, oestriol glucosiduronate and oestriol sulphate by the kidney in late human pregnancy has been compared with simultaneous measurements of inulin clearance. The results for the fractions not bound to protein indicate that after glomerular filtration, unconjugated oestriol is completely reabsorbed, oestriol glucosiduronate is both filtered and excreted actively by the tubules, and that oestriol sulphate is filtered but not excreted or absorbed by the tubules; the administration of probenecid confirms these observations on tubular handling. The possibility of using endogenous oestriol sulphate as a means of measuring the glomerular filtration rate in pregnancy is suggested.

THE excretion of oestriol in the urine is a reflection of the output of the steroid by the feto-placental unit and urinary oestriol assays have been established in clinical obstetrics as a measure of feto-placental function. The role of the maternal kidney in the excretion of oestriol is unknown and from time to time doubts have arisen as to whether feto-placental production was always the dominant factor in determining urinary oestriol output. In patients with impaired renal function it has been suggested that a low urinary oestriol output may be a reflection of impaired renal excretion and not of low oestriol production (Talbert and Easterling, 1967; Carrington *et al.*, 1970).

Before the development of radioimmunoassay techniques for the measurement of plasma steroids, studies of renal clearance of oestriol were made using total plasma oestriol and total urinary oestriol for the calculation. Brown *et al.* (1964) expressed their doubts of this approach because the bound fraction would not be filtered but suggested an oestriol clearance value of 205 ml. per minute. Talbert and Easterling (1967) and Carrington *et al.* (1970) on the other hand, gave oestriol clearances values of 95 ml. per minute and 77 ml. per minute respectively. They measured total plasma and total urinary oestriol after acid hydrolysis of all the various conjugates.

Oestriol is present in human pregnancy plasma both bound to protein and unbound, as unconjugated oestriol, oestriol-16-glucosiduronate, oestriol-3-glucosiduronate, oestriol-3-sulphate and oestriol-3-sulphate-16-glucosiduronate.

The molecular weights of oestriol and the four conjugates lie in the range 250 to 500 and with diffusion coefficients of more than 0·5 there is no reason to believe that the non-protein bound fractions will not be filtered at the glomerulus where the limit of glomerular permeability lies with molecules with diffusion coefficients of less than 0·1 (Bunim *et al.*, 1937). Protein binding is unlikely to be mechanically disrupted and any excretion of protein bound oestriol is likely to be by active tubular excretion (Smith, 1951*a*). In this study the renal clearance of oestriol and oestriol-3-sulphate were measured and compared with simultaneous inulin clearance. It was not possible to differentiate between oestriol-3-glucosiduronate, oestriol-16-glucosiduronate and oestriol-3-sulphate-16-glucosiduronate and they have been measured together as oestriol-glucosiduronate.

## METHODS

Seven healthy women in the 37th to 38th week of their pregnancy volunteered to take part in the clearance study. None had a history of renal disease. Five were primigravidae and had single pregnancies; two parous women had twin pregnancies. Each woman was admitted to the Maternity Hospital on the day before the test. Since the presence of fructose in urine would confuse the estimations of inulin, fruit and fruit juice were omitted from the diet to reduce fructose intake. Two primigravidae received probenecid 1 g. at 2200 hours on the day of admission and 500 mg. six-hourly thereafter. On the morning of the test, after obtaining urine and plasma blank samples, each subject received a loading dose of inulin and para-amino hippuric acid (PAH), and the infusion of inulin and PAH was maintained using a Harvard constant infusion syringe pump. The infusion solution contained 2·7 g. of inulin and 1·08 g. of PAH per 100 ml. of 0·9 g. per cent sodium chloride solution. The loading dose of 1·85 ml./kg. body weight was run in rapidly and the infusion was maintained at 1·5 ml. per minute. Demand voiding of urine was avoided since this may be associated with incomplete emptying of the bladder. The first urine void, usually 2 to 3 hours after the start of the infusion, was discarded and the time noted. Thereafter all urine voided was collected, measured and the time of voiding noted. Blood samples were taken three and six hours after the infusion began for oestriol, inulin and PAH determination. The test lasted for six to eight hours after the first void of urine. This method of measuring inulin clearance which has been developed in this unit, avoids the need for bladder catheterization and the results are reproducible in the same woman (Swapp, 1974).

Plasma for oestriol assay was divided and one aliquot ultra-filtered through a Centiflo Membrane Ultrafilter (American Corporation, Lexington, Mass.) to keep back particles above molecular weight 30,000 and thus produce a protein free ultrafiltrate. The protein content of the plasma and plasma ultrafiltrate was measured by the Kjeldhal distillation method.

Urine plasma and plasma ultrafiltrate were assayed by radioimmunoassay using the principles of the method developed by Gurpide *et al.* (1971) as modified by Wilson (1973). The antiserum used was obtained from sheep immunized by bovine albumin linked to oestriol-16, 17-dihemisuccinate. Free oestriol was first removed by ether extraction, the sulphate fraction obtained by solvolysis and glucosiduronate by acid hydrolysis of the remainder. Oestriol was separated from oestrone and oestrediol 17$\beta$ by partition between petroleum ether-benzene and water before radioimmunoassay. Inulin and PAH in plasma and urine were estimated by the method described by Varley (1962).

## RESULTS

The concentrations of unconjugated oestriol, oestriol-glucosiduronate and oestriol-3-sulphate in plasma, plasma ultrafiltrate and urine are shown in Table I. No unconjugated oestriol was found in the urine except when patients were treated with probenecid (subjects F and G). The molecular weight of unconjugated oestriol is 286 and molecules of this size can readily be filtered at the renal glomerulus; its presence in the ultrafiltrate of plasma indicates that it is present in plasma unbound to protein and is therefore available for filtration.

The two conjugates of oestriol studied are present in the plasma ultrafiltrate and urine indicating that they are available for glomerular filtration and have been excreted in the urine.

In Table II clearance values for inulin, PAH, unconjugated oestriol, oestriol-glucosiduronate, and oestriol-3-sulphate are given and the clearance ratios with inulin have been calculated for oestriol-glucosiduronate and oestriol-3-sulphate. For subjects A, B, C, D and E the clearance of oestriol-glucosiduronate is much greater than the simultaneous inulin clearance. The administration of probenecid has reduced the clearance

TABLE I

*Concentrations of oestriol* ($E_3$); *oestriol-glucosiduronate* (EG); *and oestriol-3-sulphate* ($E_3S$) *in plasma, plasma ultrafiltrate and urine*

| Subject | Plasma (μg. EG/100 ml. $E_3S$) | | | Plasma ultrafiltrate (μg. EG/100 ml. $E_3S$) | | | Urine (μg. EG/100 ml. $E_3S$) | | |
|---|---|---|---|---|---|---|---|---|---|
| A | 2·12 | 12·71 | 12·81 | 1·37 | 4·99 | 2·99 | 0·0 | 374 | 137 |
| B | 2·52 | 13·79 | 16·51 | 0·70 | 2·65 | 2·45 | 0·0 | 334 | 106 |
| C | 6·18 | 13·99 | 12·58 | 1·69 | 9·04 | 3·39 | 0·0 | 506 | 114 |
| D | 1·80 | 8·57 | 6·11 | 0·60 | 3·55 | 2·63 | 0·0 | 350 | 110 |
| E | 2·51 | 20·21 | 8·86 | 1·05 | 3·34 | 4·16 | 0·0 | 204 | 67 |
| *F | 7·4 | 20·1 | 7 8 | 0·60 | 8·6 | 1·5 | 15·8 | 540 | 81 |
| *G | 7·3 | 11·0 | 15·2 | 1·20 | 8·7 | 1·8 | 28·5 | 519 | 60 |

* Subjects F and G received probenecid.

TABLE II

*Plasma ultrafiltrate clearance values* (C) *for insulin* (I), *P.A.H., oestriol* ($E_3$), *oestriol-glucosiduronate* (EG), *oestriol-3-Sulphate* ($E_3S$), *and Clearance Ratios*

| Subject | Urine flow (ml./minute) | Clearance values (ml./per minute) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | $C_I$ | $C_{PAH}$ | $C_{E_3}$ | $C_{EG}$ | $C_{E_3S}$ | $C_{EG}/C_I$ | $C_{E_3S}/C_I$ |
| A | 3·32 | 163 | 779 | 0 | 248 | 151 | 1·5 | 0·9 |
| B | 2·8 | 131 | | 0 | 356 | 122 | 2·7 | 0·93 |
| C | 3·54 | 140 | 547 | 0 | 198 | 119 | 1·4 | 0·85 |
| D | 2·5 | 102 | 504 | 0 | 246 | 104 | 2·4 | 1·02 |
| E | 3·4 | 94 | 577 | 0 | 208 | 55 | 2·2 | 0·6 |
| F | 2·1 | 170 | 614 | 55·3 | 131·6 | 113·8 | 0·78 | 0·7 |
| G | 2·1 | 92 | 658 | 50·6 | 125·0 | 71·6 | 1·4 | 0·8 |

and the clearance ratio indicating that tubular excretion has been partly blocked by the probenecid.

The clearance of oestriol-3-sulphate and its clearance ratio with inulin suggest that, after filtration it is neither reabsorbed nor excreted by the tubules. This observation is confirmed in subjects F and G who received probenecid.

In Table III the clearance values for oestriol and the two conjugates have been calculated using whole plasma values. The effect of not allowing for protein binding would give the impression that both conjugates were re-absorbed by the tubules.

TABLE III

*Whole plasma clearance* (C); *values for oestriol* ($E_3$); *oestriol-glucosiduronate* (EG); *and oestriol-3-sulphate* ($E_3S$)

| Subject | Clearance values (ml./minute) | | |
|---|---|---|---|
| | $C_{E_3}$ | $C_{EG}$ | $C_{E_3S}$ |
| A | 0 | 100 | 35 |
| B | 0 | 68 | 18 |
| C | 0 | 128 | 32 |
| D | 0 | 102 | 45 |
| E | 0 | 34 | 26 |
| F | 4 | 56 | 22 |
| G | 8 | 99 | 8 |

## DISCUSSION

A standard of reference suitable for measuring glomerular filtration rate must fulfill certain specifications: inulin is filtered at the glomerulus and is not reabsorbed or excreted by the renal tubules and thus is a measure of the glomerular filtration rate. More important, simultaneous clearance studies with inulin can indicate whether the filtrable substance being studied is reabsorbed or excreted by the renal tubules in addition to being filtered at the glomerulus (Smith, 1951*b*).

The molecular weights and diffusion coefficients of oestriol and the conjugates studied are such that the plasma fractions not bound to protein will be filtered by the renal glomerulus. The protein bound fraction will not be filtered by the healthy glomerulus and in none of our subjects was there a history of renal disease or evidence of renal disease.

Unconjugated oestriol, oestriol-3-sulphate, oestriol-glucosiduronate were all present in the protein free filtrate of plasma indicating that they exist in an unbound form in plasma and are available for glomerular filtration. Unconjugated oestriol was not detected in the urine and this means that after filtration it has been reabsorbed by the renal tubules. Oestriol-glucosiduronate was present in the urine and the clearance ratio with inulin in each subject was more than 1·0 (Table II) indicating that in addition to glomarular filtration, tubular excretion had taken place. Oestriol-3-sulphate was present in the urine and the clearance ratio with inulin was about 1·0 (Table II) indicating that after filtration it was probably neither reabsorbed or excreted by the renal tubules.

The effect of administrating probenecid, which blocks the tubular excretion and reabsorption of a wide range of substances (Smith, 1956) is shown in Table I and II (Subjects F and G). Unconjugated oestriol appears in the urine and this confirms the observation that it had been actively reabsorbed after glomerular filtration. The clearance of oestriol sulphate is unchanged indicating that tubular reabsorption or excretion play no part in its renal excretion which appears to be by glomerular filtration only. This observation may be of value in that endogenous oestriol-3-sulphate clearance measurements may be used instead of inulin in clearance studies in normal pregnancy. Oestriol-glucosiduronate clearance is lowered by probenecid administration indicating that its tubular excretion is probably an active process.

Protein binding does not render a substance unavailable for tubular excretion. If the tubules take up unbound conjugate from the interstitial fluid, diffusion from the tubular capillaries will reduce the plasma concentration and promote dissociation of the protein complex (Smith, 1951*a*).

Kirdani *et al.* (1972) have demonstrated by direct renal perfusion studies and incubation of homogenates of human kidney that glucosiduronation of unconjugated oestriol can take place. The clearance of oestriol-glucosiduronate is less than the PAH clearance, i.e. it is less than the renal plasma flow. This indicates that even with tubular excretion renal extraction is incomplete (Smith, 1951*c*). Had the clearance been greater than the PAH clearance it would have suggested active synthesis of the conjugate by the kidney.

These observations show the importance of allowing for protein binding in clearance studies and of differentiating between the clearances of oestriol and its conjugates. In renal disease where proteinuria is present or perhaps tubular function is impaired the clearance values may be quite different and measurements of urinary oestriol may reflect maternal renal impairment as well as fetoplacental function. Roy *et al.* (1964) have suggested impaired renal excretion of oestriol in pre-eclampsia.

Further studies of this problem will be facilitated by the development of methods to differentiate the two glucosiduronate molecules of oestriol and oestriol-3-sulphate-16-glucosiduronate. These are being actively developed.

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# ASSESSMENT OF THYROID FUNCTION DURING PREGNANCY

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## Summary

Serum levels of thyroid stimulating hormone and of the two thyroid hormones were compared in pregnant women and in non-pregnant control subjects. Circulating levels of thyroid stimulating hormone did not alter significantly throughout pregnancy. Total thyroxine and triiodothyronine concentrations increased markedly during the first and second trimesters, associated with a progressive increase in the thyroid binding capacity of serum proteins. Urinary triiodothyronine and thyroxine excretion during the third trimester of pregnancy was comparable to that found in non-pregnant euthyroid females and, it is concluded, provides the simplest method of assessing thyroid function during pregnancy.

THE recognition of thyroid dysfunction in a pregnant patient is important for the welfare of both mother and fetus. Clinical assessment of thyroid status is, however, difficult because physiological changes associated with normal pregnancy may mimic the signs and symptoms of hypo- or hyperthyroidism. Biochemical assessment is also difficult due to changes in the results of various thyroid function tests, such as protein bound iodine (Heineman *et al.*, 1948), total thyroxine ($T_4$) (Malkasian and Mayberry, 1970), total triiodothyronine ($T_3$) (Hotelling and Sherwood, 1971) and the thyroid hormone uptake test (THUT) (Hollander *et al.*, 1963; Winikoff and Taylor, 1966), due to an increase in certain serum proteins and in their capacity to bind thyroid hormones (Dowling *et al.*, 1956; Engbring and Engstrom, 1959; Tata, 1960). In these circumstances, the ideal laboratory test is the measurement of circulating free $T_3$ and $T_4$, as it is probably only the free fractions which are biologically active and reflect the true thyroid status of the individual. Unfortunately, available direct techniques are time-consuming and subject to a variety of artefacts (Spaulding and Gregerman, 1972). An alternative approach, involving the calculation of the free thyroxine index (FTI) (Clark and Horn, 1965) has proved unsatisfactory, as it is not proportional to free $T_4$ in the second and third trimester (Souma *et al.*, 1973) and it provides us no information about circulating $T_3$ which is of equal, if not more, importance. A second indirect approach involves the determination of the urinary excretion of $T_3$ and $T_4$ which normally relate closely to circulating free hormone concentrations (Chan and Landon, 1972; Chan *et al.*, 1972; Burke *et al.*, 1972; Chan, 1974; Burke and Shakespeare, 1974).

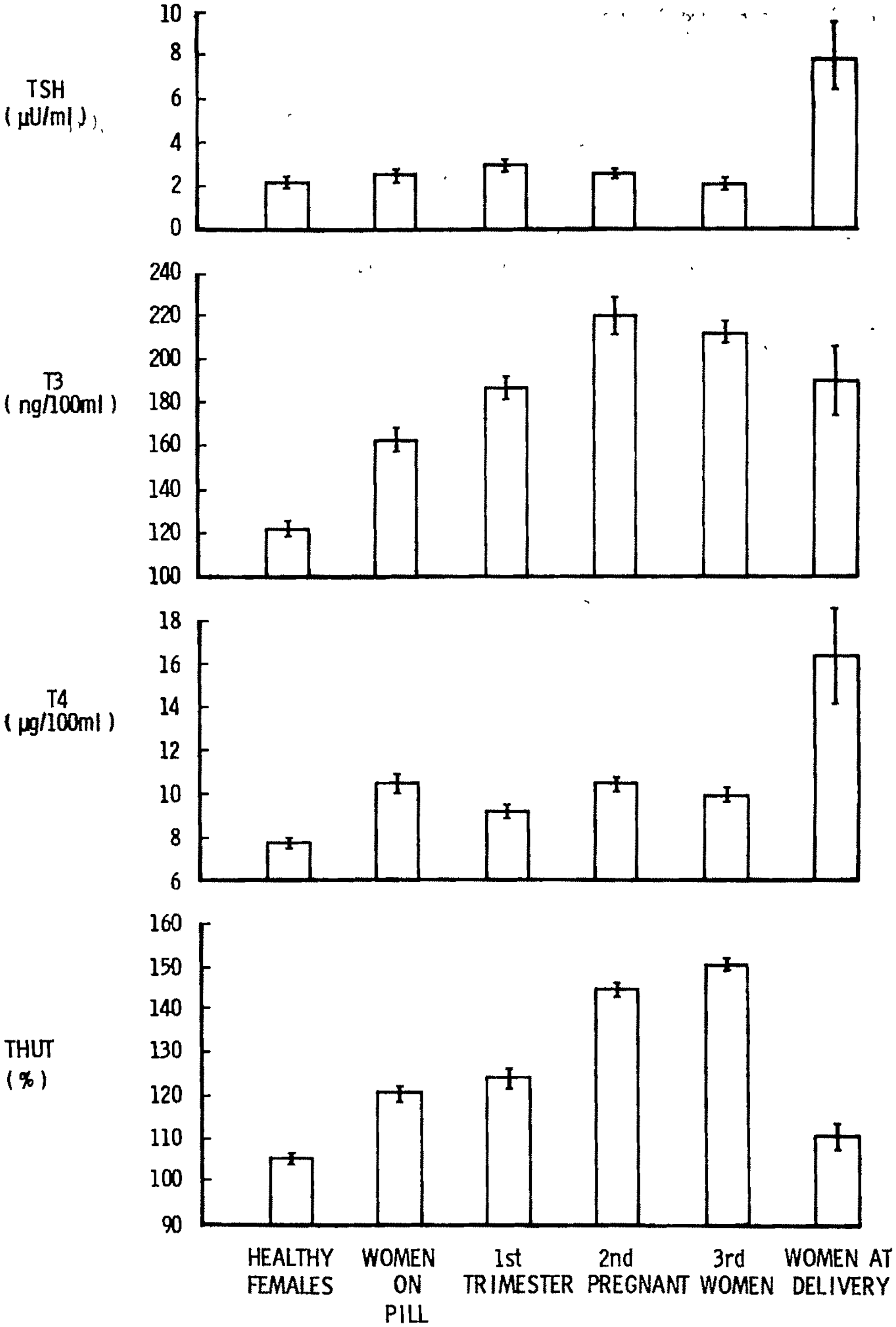

Fig. 1

Mean values (±SEM) for circulating thyrotrophin (TSH), thyroid hormone and thyroid hormone uptake test (THUT) in non-pregnant females, pregnant women, women at term and women on combined oestrogen-progestogen oral contraceptives.

The purpose of the present study was to investigate the conventional serum thyroid function tests with a view to establishing a normal range for each trimester of pregnancy, and to compare the results obtained with the urinary thyroid hormone levels found in non-pregnant euthyroid females.

## METHODS

A total of 303 subjects was studied, comprising 91 non-pregnant women, 41 of whom had been receiving combined oestrogen-progestogen oral contraceptives for three or more months, eight women immediately after delivery, and 204 pregnant women, of whom 51 were in the first, 50 in the second, and 103 in the third trimester. All were healthy, without previous history of thyroid disease and were not taking any drugs (other than oral contraceptives) known to affect thyroid function tests.

A 4 ml. blood sample was taken from each subject, the serum separated within 2 hours and stored frozen until required for assay. In addition, 24-hour urine collections were made by 24 euthyroid non-pregnant women and 24 women in the third trimester of pregnancy.

Total $T_3$ concentrations were determined in 50 $\mu$l. of whole serum by a highly sensitive and precise radioimmunoassay (De Souza *et al.*, 1974) using 8-anilino-1-naphthalene sulphonic acid to inhibit $T_3$ binding to endogenous thyroxine binding globulin (TBG), and charcoal to separate the antibody bound and free hormone fractions. The anti-$T_3$ serum was raised in goats by multiple intradermal injections of a $T_3$-bovine serum albumin conjugate and cross-reacted with $T_4$ only to the extent of 0·0125 per cent.

Serum total $T_4$ was measured by a competitive protein binding assay using the Thyopac-4 kit (Radiochemical Centre, Amersham) and the thyroid hormone uptake test (THUT), which measures the degree of unsaturation of thyroid binding proteins, was determined using the Thyopac-3 kit (Radiochemical Centre, Amersham). The FTI was calculated from the results of these two tests.

Circulating TSH levels were measured by a modification of the double-antibody radioimmunoassay method of Hall and his colleagues (1971), and the results are expressed as $\mu$U/ml. of the MRC Research Standard 68/38. The antiserum used was raised in rabbits against porcine TSH and was highly specific, showing no cross-reactivity with human chorionic gonadotrophin.

Urinary $T_3$ and $T_4$ levels were measured as described elsewhere (Chan, 1974) and the results expressed as $\mu$g. of hormone excreted per 24 hours.

## RESULTS

Mean values for serum TSH, total $T_3$, $T_4$ and THUT in control subjects, women on combined oral contraceptives, women in each trimester of pregnancy and in maternal sera taken after completion of the second stage of labour are presented in Figure 1 and Table I. The binding capacity of TBG, as determined by the thyroid hormone uptake test, increased progressively during pregnancy and was accompanied by increases in circulating total $T_3$ and $T_4$ levels in the first and second trimester. The mean $T_3$ (211±55 ng./100 ml.) and $T_4$ (9·99±2·07 $\mu$g./100 ml.) values in the third trimester were slightly, though not significantly, lower than those in the second trimester. Serum TSH values were the same in all groups, except for a small but insignificant increase ($p<0\cdot1>0\cdot05$), from 2·16±1·09 $\mu$U/ml. in the control group, to 2·87±2·05 $\mu$U/ml. in the first trimester. Much higher levels were recorded in maternal sera at the end of the second stage of labour (mean 7·78 $\mu$U/ml.).

The FTI was highest in the first trimester (8·11±1·86) and decreased by the third trimester to 7·24±1·45, which is lower than that of the control group (7·76±1·85). A significantly higher FTI was found at the time of delivery ($p<0\cdot001$).

Circulating $T_3$ and $T_4$ levels, the THUT and the FTI were elevated in women on combined oral contraceptives as compared with the control group, while their total $T_3$ and THUT were lower, and their $T_4$ and FTI higher than in women in the first trimester. Serum TSH levels remained normal in women on combined oral contraceptives.

In the 24 third trimester patients studied, urinary $T_3$ and $T_4$ excretion remained in the accepted normal range, with similar mean values to those of 24 non-pregnant euthyroid females (Fig. 2).

TABLE I

*Serum TSH and thyroid hormone levels in pregnant women, women at delivery, women on oestrogen-progestogen, and euthyroid non-pregnant women*

| | Healthy euthyroid women (50) | Pregnant women | | | Women at delivery (8) | Women on oestrogen–progestogen (41) |
|---|---|---|---|---|---|---|
| | | First trimester (51) | Second trimester (50) | Third trimester (103) | | |
| *TSH (μU/ml.)* | | | | | | |
| Mean ± SD | 2·2±1·1 | 2·9±2·1 | 2·6±2·2 | 2·1±1·7 | 7·8±1·1 | 2·5±1·5 |
| SEM | 0·17 | 0·32 | 0·35 | 0·20 | 0·38 | 0·24 |
| Calculated range* | <0·2–4·3 | <0·2–7·0 | <0·2–7·1 | <0·2–5·6 | 5·6–9·9 | <0·2–5·6 |
| Observed range | 0·4–4·8 | <0·2–10·2 | <0·2–7·2 | <0·2–7·5 | 4·1–10·5 | <0·2–4·8 |
| *THUT (per cent)* | | | | | | |
| Mean ± SD | 106±6·8 | 125±12·4 | 145±9·7 | 151±9·6 | 111±3·3 | 121±9·7 |
| SEM | 0·95 | 1·73 | 1·46 | 1·03 | 1·17 | 1·51 |
| Calculated range* | 92–120 | 100–149 | 126–165 | 132–171 | 104–118 | 102–140 |
| Observed range | 84–120 | 96–148 | 124–164 | 131–173 | 96–124 | 102–136 |
| $T_4$ *(μg./100 ml.)* | | | | | | |
| Mean ± SD | 7·8±1·6 | 9·2±2·3 | 10·5±1·7 | 10·0±2·1 | 16·2±2·1 | 10·5±3·0 |
| SEM | 0·22 | 0·33 | 0 26 | 0·22 | 0·74 | 0·44 |
| Calculated range* | 4·7–10·9 | 4·7–13 8 | 7·1–13·9 | 5·9–14·1 | 12·0–20·4 | 4·6–16·4 |
| Observed range | 5·4–12·8 | 4·5–15·8 | 6·7–13·9 | 6·8–15·4 | 9·0–28·0 | 6 4–17·4 |
| *FTI* | | | | | | |
| Mean ± SD | 7·8±1·9 | 8·1±1·9 | 7·9±1 3 | 7·2±1·5 | 16 5±2·9 | 8·7±2·7 |
| SEM | 0·26 | 0·26 | 0·20 | 0·14 | 1·03 | 0·41 |
| Calculated range* | 4·1–11·5 | 4·4–11·8 | 5·4–10·5 | 4·3–10·1 | 10·7–22·3 | 3 4–14·0 |
| Observed range | 5·4–12·1 | 4·9–15·3 | 5·5–10·7 | 3·0–10·8 | 9 4–34·2 | 5·4–15·8 |
| $T_3$ *(ng./100 ml.)* | | | | | | |
| Mean ± SD | 121±20 | 187±40 | 220±56 | 212±56 | 191±8 | 163±29 |
| SEM | 2·8 | 5·5 | 7·9 | 5·5 | 2·9 | 4·5 |
| Calculated range* | 81–161 | 107–268 | 102–338 | 100–323 | 175–207 | 105–221 |
| Observed range | 81–159 | 74–280 | 100–364 | 81–335 | 163–219 | 90–220 |

* Mean ± 2SD.

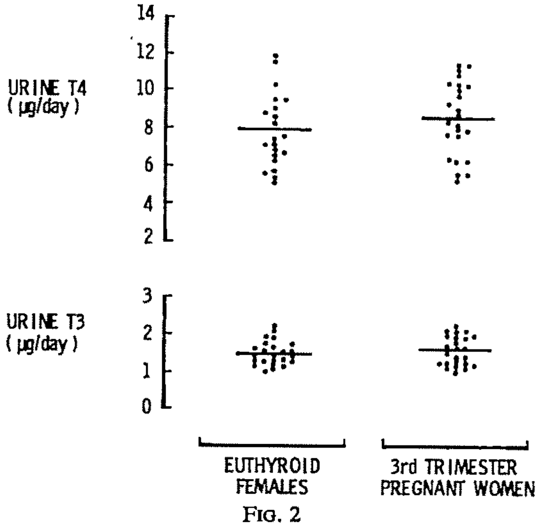

FIG. 2

Urinary $T_3$ and $T_4$ levels in healthy euthyroid females and women in the third trimester of pregnancy. Mean values are represented by the horizontal lines.

## DISCUSSION

It is well recognized that increased levels of oestrogen in the blood, whether of endogenous or exogenous origin, are associated with an increase in TBG and a corresponding increase in total $T_4$ (Malkasian and Mayberry, 1970). Controversy exists, however, regarding the concentrations of circulating $T_3$ and TSH in pregnancy. Dussault *et al.* (1969) found no change in $T_3$ concentrations while Hotelling and Sherwood (1971), studying 12 women in each trimester of pregnancy, found that $T_3$, like $T_4$, increased progressively to maximal levels at term. Our study, involving a much larger number of subjects, shows that both $T_3$ and $T_4$ are increased and are maximal during the second trimester.

In agreement with others (Odell *et al.*, 1963; Freychet *et al.*, 1969), we find that serum TSH levels remain normal during pregnancy. Genazzani and his colleagues (1971) reported elevated TSH levels from the seventh week of pregnancy until 30 minutes after delivery, but concluded that this was probably due to the presence of a placental thyrotrophin-like peptide which cross-reacted with their antiserum. It would seem that our TSH antiserum does not cross-react with human chorionic TSH. The high TSH levels found in maternal sera at delivery may indicate a pituitary response to labour, although we have not excluded the possible effects of drugs given at this time. The significance of this finding, and of the rise in total $T_4$ and fall in THUT during labour, merit further investigation.

Souma *et al.* (1973) demonstrated that the FTI fell during the course of pregnancy while circulating free $T_4$ remained relatively stable, and similar to the levels found in non-pregnant controls. In our experience, urinary $T_3$ and $T_4$ concentrations remain normal in pregnant women, indicating that these measurements are not affected by changes in the TBG concentration or its binding capacity. As urinary thyroid hormone levels correlate closely with circulating free hormone concentrations (Burke and Shakespeare, 1974) and specific radioimmunoassays can be performed on urine directly, this would appear, at present, to be the most convenient test of thyroid function during pregnancy.

## ACKNOWLEDGEMENTS

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# METABOLISM OF PROSTAGLANDIN $F_{2\alpha}$ WITHIN THE HUMAN UTERUS IN EARLY PREGNANCY

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## Summary

The potential for intra-uterine metabolism of prostaglandins in early pregnancy was studied by incubation of prostaglandin $F_2\alpha$ ($PGF_2\alpha$) with tissues obtained by termination hysterectomy at 7 and 16 weeks gestation. Enzymes regulating the degradation of prostaglandins were demonstrated in placenta and membranes and to a lesser extent, in myometrium and decidua. It is believed that these enzymes may have a physiological rôle in maintaining the continuity of pregnancy, and that the high degradation of $PGF_2\alpha$ in placenta and membranes may be one of the reasons that, for termination of pregnancy, far higher doses are required intra-amniotically than extra-amniotically.

IT is now common knowledge that naturally occurring prostaglandins are metabolized by a chain of reactions which, in man, starts with oxidation at carbon atom 15 by the enzyme 15-hydroxy-prostaglandin dehydrogenase (Samuelsson *et al.*, 1971). The recognition of the initial step in the degradation of prostaglandins has led to the synthesis of 15-methyl analogues of the natural prostaglandins which are resistant to degradation by 15-hydroxy-prostaglandin dehydrogenase (Bundy *et al.*, 1971; Yankee and Bundy, 1972). Since natural prostaglandins are rapidly inactivated on perfusion through the lungs (Ferreira and Vane, 1967; Piper *et al.*, 1970), it is perhaps not surprising that their 15-methyl analogues display a higher potency and a longer duration of action than the parent compounds (Kirton and Forbes, 1972; Karim and Sharma, 1972).

These analogues also proved to be far more active than the natural compounds, when administered locally (intra- or extra-amniotically) for the termination of pregnancy (Bygdeman *et al.*, 1972; Karim and Sharma, 1972; Amy *et al.*, 1973). This could suggest that there is a potential for intra-uterine metabolism of natural prostaglandins in the second trimester of pregnancy, and that the large doses of natural prostaglandins required for the termination of pregnancy may be dictated partially by the need to overcome or compensate for their degradation. To test this hypothesis, we investigated, *in vitro*, the capacity of various intra-uterine tissues to metabolize a prostaglandin.

## METHODS

A complete uterus containing an intact gestational sac, was obtained by total hysterectomy from each of two patients, at 7 and 16 weeks gestation respectively. In both cases pregnancy was terminated on social grounds, and no pathology, other than fibroids, was found. At 16 weeks gestation, placental villous tissue membranes, umbilical cord, fetal skin, macroscopically normal myometrium and decidua were investigated whilst at the earlier gestation only normal myometrium and villous tissue were investigated.

The method used was that described by Keirse and Turnbull (1974). All tissues were homogenized and the homogenates incubated with $^3H$-prostaglandin $F_{2\alpha}$ ($^3H$-$PGF_{2\alpha}$; 0·9 μg./g. of tissue) in 0·1M potassium phosphate buffer, pH 7·4, containing 2 mM. nicotinamide adenine dinucleotide (NAD) at 37 °C. for various lengths of time. A placental homogenate, previously inactivated by boiling, was used for the "control" incubation. After the reactions had been stopped by acidifying and addition of ethanol, $PGF_{2\alpha}$ was extracted and separated from its metabolites by thin layer chromatography. The plates were scanned for radioactivity using a radiochromatogram scanner, and the relevant zones were eluted for quantitative determination of radioactivity by liquid scintillation counting.

## RESULTS

At 16 weeks gestation there was considerable metabolism of $PGF_{2\alpha}$ by the membranes and placental homogenates; $PGF_{2\alpha}$ concentrations dropped to less than half their original concentration (0·9 μg./g. of tissue) within three minutes, as shown in Figure 1. Myometrial and decidual homogenates were less active, metabolizing respectively 15 and 5 per cent of the $PGF_{2\alpha}$ in 30 minutes. The fetal skin and umbilical cord, on the other hand, appeared to be devoid of enzymatic activity since the $PGF_{2\alpha}$ concentrations after incubation up to 60 minutes were not different from those in the control incubation with boiled homogenate.

The results obtained with myometrium and villous tissue at 7 weeks gestation were similar to those observed at 16 weeks, showing that prostaglandin metabolizing enzymes are present in the placenta as early as the 7th week of pregnancy.

Differentiation of the metabolites by thin layer chromatography showed that $PGF_{2\alpha}$ was metabolized to at least two compounds. Using an incubation of membranes as an example, the distribution of the total radioactivity between $PGF_{2\alpha}$ and these metabolites ($M_1$ and $M_2$) after various lengths of time, is shown in Figure 2. $M_1$ and $M_2$ showed a mobility on thin layer chromatography identical respectively to that

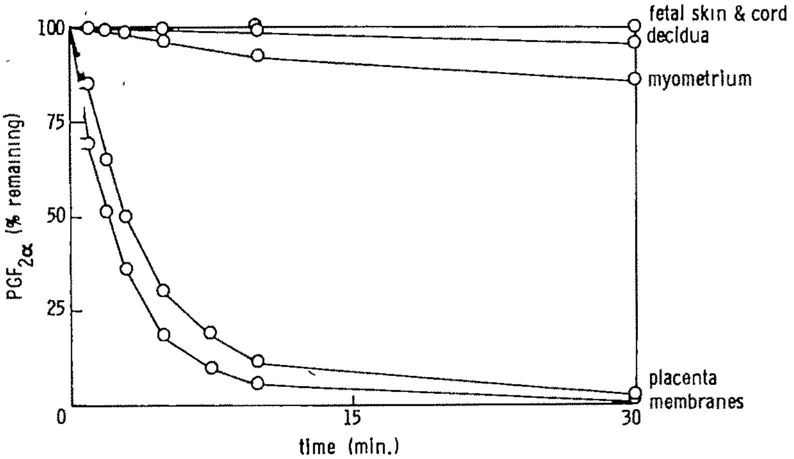

FIG. 1

Metabolic degradation of $PGF_{2\alpha}$ in homogenates of fetal skin, umbilical cord, decidua, myometrium, placenta, and fetal membranes obtained at 16 weeks gestation.

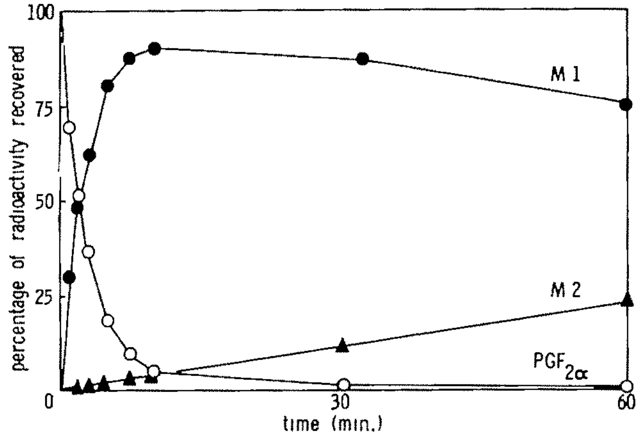

FIG. 2
Distribution of the radioactivity between PGF$_{2\alpha}$ and its metabolites (M$_1$ and M$_2$) during incubation of 3H-PGF$_{2\alpha}$ with a homogenate of membranes, obtained at 16 weeks gestation.

of 15-keto PGF$_{2\alpha}$ and 15-keto-13,14-dihydro PGF$_{2\alpha}$.

## DISCUSSION

The observations made in this study demonstrate the presence of enzymes regulating the degradation of prostaglandins, in placenta and membranes and to a lesser extent in myometrium and decidua, in early pregnancy. Since both E and F prostaglandins are substrates for 15-hydroxy-prostaglandin dehydrogenase (Änggård and Samuelsson, 1966), the present observations using PGF$_{2\alpha}$ as a substrate can also be applied to PGE$_2$. In fact, PGE is generally metabolized faster than PGF in the *in vitro* situation (Änggård and Samuelsson, 1966; Jarabak, 1972; Schlegel *et al.*, 1974).

The placenta has previously been shown to contain a high prostaglandin dehydrogenase activity *in vitro* (Jarabak, 1972; Schlegel *et al.*, 1974). The present study, however, demonstrates that the enzymatic activity is present in villous tissue as early as the 7th week of pregnancy, and that this activity is not limited to placental tissue.

The present findings certainly support the hypothesis that there is a high potential for intra-uterine metabolism of prostaglandins in the second trimester of pregnancy. The extent to which this potential, observed *in vitro*, applies *in vivo* is not known. It is interesting, however, that the myometrium is divided from the amniotic cavity by two structures of fetal origin, membranes and placenta, which showed a remarkably high degradation of prostaglandins *in vitro*. Prostaglandins administered intra-amniotically for termination of pregnancy have been shown to disappear steadily from the amniotic cavity (Pace-Asciak *et al.*, 1972) and Karim (1972) has suggested that they diffuse through the membranes to reach their site of action in the myometrium. It is almost certain that a proportion of these prostaglandins must be inactivated before reaching the myometrial target and this may well be one of the reasons that, for termination of pregnancy, far higher doses of prostaglandins are required intra-amniotically than extra-amniotically (Wiqvist *et al.*, 1973).

Prostaglandins have been implicated as causative agents in the process of spontaneous and habitual abortion (Karim and Hillier, 1970; Bodin, 1971). It is possible that the physiological rôle of the prostaglandin-degrading enzymes is to maintain the continuity of pregnancy. The fact that the highest enzyme activities were found in fetal tissues, membranes and placenta, may provide yet another argument for a fetal rôle in the maintenance of pregnancy.

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# MORPHOLOGICAL CHANGES IN MATERNAL LYMPHOCYTES IN PREGNANCY

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## Summary

By use of staining with toluidine blue, the changes in the nucleoli of lymphocytes in 150 healthy women were studied. The patients were divided into five groups: non-pregnant women; women in the first, second and third trimesters of pregnancy; and women who were five days *post partum*. A progressive and statistically significant rise in the number of lymphocytes with micronucleoli was found during pregnancy and there was a fall after delivery. This increase was associated with a decrease in the incidence of ring-shaped nucleoli. There were no significant changes in the absolute number of lymphocytes and in "active" lymphocytes during pregnancy.

MORPHOLOGICAL changes in lymphocytes are usually studied during maturation of these cells or after stimulation with different antigens. Maturation is accompanied by gradual nucleolar changes characterized by the presence of ring-shaped nucleoli or micronucleoli. The microscopic studies of nucleoli by Smetana (1961) and Potměšil and Smetana (1969) revealed changes related to the inhibition of ribonucleic acid (RNA) synthesis and formation of nucleolar riboprotein structures that enabled them to divide the cells according to the character of nucleoli into four groups: compact nucleoli, nucleoli with nucleolonemas, ring-shaped nucleoli and micronucleoli (Figs. 1, 2 and 3).

Previous studies suggested that the lymphocytes with compact nucleoli or nucleolonemas were "active" and produced RNA. Ring-shaped nucleoli might represent reversible and micronucleoli irreversible nucleolar changes induced by the inhibition of RNA synthesis, which occurred during the cell maturation or under special experimental conditions.

After phytohaemagglutinin (PHA) stimulation, in infectious diseases, or after a transplantation of a graft an increase of active lymphocytes was found, a shift related to the fall of ring-shaped nucleoli.

We have not found any paper dealing with changes in lymphocytic nucleoli during pregnancy, though the contact between mother and fetus may be very similar to the interaction between graft and host where the reaction is mediated by lymphocytes. This study attempts to find out if pregnancy alone might cause any morphological changes in the lymphocytic nucleoli.

## METHODS

Five groups, each of 30 healthy women, were studied. The first group comprised non-pregnant women, 19 of them nulligravidae. The second,

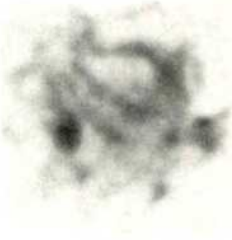

FIG. 1
Lymphocyte with compact nucleolus.
(Toluidine blue × 1000).

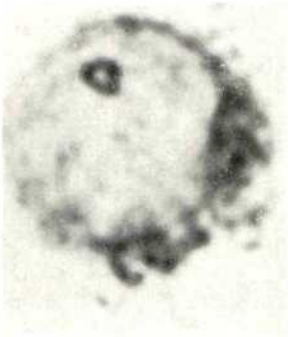

FIG. 2
Lymphocyte with ring-shaped nucleolus.
(Toluidine blue × 1000.)

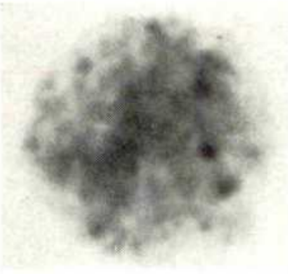

FIG. 3
Lymphocyte with micronucleoli.
(Toluidine blue × 1000).

third and fourth groups of women were in the first, second and third trimesters of normal pregnancy respectively. The last group comprised women who had had a spontaneous delivery five days previously. In all, 42 of these 120 women were primigravidae. The average age was 23·6 years.

Peripheral blood samples were collected for routine haematological examinations, and an extra blood smear was stained with toluidine blue according to the method of Smetana (1961). Within seven days 200 lymphocytes in each smear were evaluated according to the morphology of the nucleoli. The technician was not informed of the classification of the patients into experimental groups. From the differential count of the leucocytes the absolute count of lymphocytes was calculated per cu. mm. Statistical analysis of the results was carried out by Student's "t"-test.

## RESULTS

Table I records the mean values for white blood cell counts, percentages of lymphocytes and absolute counts of lymphocytes per cu. mm. There was a significant increase of leucocytes post partum compared with the non-pregnant women ($p<0\cdot01$), and with pregnant women in the first and second trimesters of pregnancy ($p<0\cdot05$). Women in the first, second and third trimesters of pregnancy and women after delivery showed a lower percentage of lymphocytes than non-pregnant women ($p<0\cdot01$). Absolute numbers of lymphocytes in pregnant women in the first and third trimesters were slightly less than in postpartum patients ($p<0\cdot05$).

Figure 4 and Table II show the appearance of nucleoli in all groups of patients. The first column of each group shown in Figure 4 shows the incidence of lymphocytes in which compact nucleoli or nucleoli with nucleolonemas were seen either alone or together with ring-shaped nucleoli: there was no significant difference between groups. The second column of each group of columns in Figure 4 shows the incidence of lymphocytes with ring-shaped nucleoli: each group differed significantly from the others ($p<0\cdot01$). The third column of each group in Figure 4 shows the incidence of lymphocytes with ring-shaped nucleoli together with micronucleoli: no statistical differences were found between the non-pregnant women and women in the first trimester or between those in the second and third trimesters, but the other relations were statistically significant ($p<0\cdot01$). The fourth column of each group in Figure 4 shows the incidence of lymphocytes with micronucleoli only: each group differed from the others ($p<0\cdot01$), but in the second trimester the difference was smaller in comparison with the postpartum group ($p<0\cdot05$).

TABLE I

*Mean white blood cell and lymphocyte counts in non-pregnant and pregnant women. The figures in parentheses indicate 95 per cent confidence limits*

| Measurement | | Non-pregnant (30 subjects) | First trimester (30 subjects) | Second trimester (30 subjects) | Third trimester (30 subjects) | Fifth day post partum (30 subjects) |
|---|---|---|---|---|---|---|
| White cells (number per cu. mm.) | Mean | 6733 | 7542* | 7596 | 7928† | 8755 |
| | | (5860–7606) | (6692–8393) | (6878–8314) | (7073–8782) | (7919–9590) |
| | S.E. | 426·84 | 407·69 | 351·14 | 414·07 | 408·03 |
| Lymphocytes (per cent of white cells) | Mean | 28·4 | 21·8 | 23·2 | 20·5 | 22·5 |
| | | (27·4–29·4) | (19·5–24·0) | (21·5–25·0) | (18·2–22·7) | (21·0–24·1) |
| | S.E. | 0·48 | 1·12 | 0·86 | 1·10 | 0·76 |
| Lymphocytes (number per cu. mm.) | Mean | 1903 | 1644* | 1767 | 1628† | 1976 |
| | | (1673–2133) | (1371–1884) | (1548–1985) | (1366–1828) | (1743–2186) |
| | S.E. | 112·35 | 123·00 | 107·03 | 111·81 | 108·32 |

* n = 21.
† n = 25.

TABLE II

*The appearance of lymphocyte nucleoli in non-pregnant and pregnant women. The figures in parentheses indicate 95 per cent confidence limits*

| Morphological characteristic | | Non-pregnant (30 subjects) | First trimester (30 subjects) | Second trimester (30 subjects) | Third trimester (30 subjects) | Fifth day post partum (30 subjects) |
|---|---|---|---|---|---|---|
| Compact nucleoli and nucleolonemas (per cent of cells examined) | Mean | 0·86 | 0·96 | 1·10 | 1·16 | 1·03 |
| | | (0·59–1·13) | (0·84–1·08) | (1·03–1·16) | (0·72–1·60) | (0·80–1·26) |
| | S.E. | 0·13 | 0·05 | 0·03 | 0·21 | 0·11 |
| Ring-shaped nucleoli (per cent of cells examined) | Mean | 80·3 | 76·5 | 60·9 | 50·4 | 54·1 |
| | | (79·2–81·4) | (74·2–78·8) | (58·1–63·7) | (48·3–52·5) | (52·6–55·5) |
| | S.E. | 0·53 | 1·12 | 1·37 | 1·03 | 0·72 |
| Ring-shaped nucleoli with micronuclei (per cent of cells examined) | Mean | 6·2 | 5·5 | 10·7 | 10·9 | 14·1 |
| | | (5·6–6·8) | (4·9–6·1) | (9·6–11·7) | (9·7–12·0) | (12·8–15·3) |
| | S.E. | 0·28 | 0·31 | 0·53 | 0·56 | 0·61 |
| Micronucleoli (per cent of cells examined) | Mean | 12·8 | 16·5 | 27·0 | 37·3 | 30·9 |
| | | (11·9–13·7) | (14·6–18·4) | (25·0–29·0) | (35·7–39·0) | (28·7–33·2) |
| | S.E. | 0·45 | 0·93 | 0·99 | 0·80 | 1·11 |

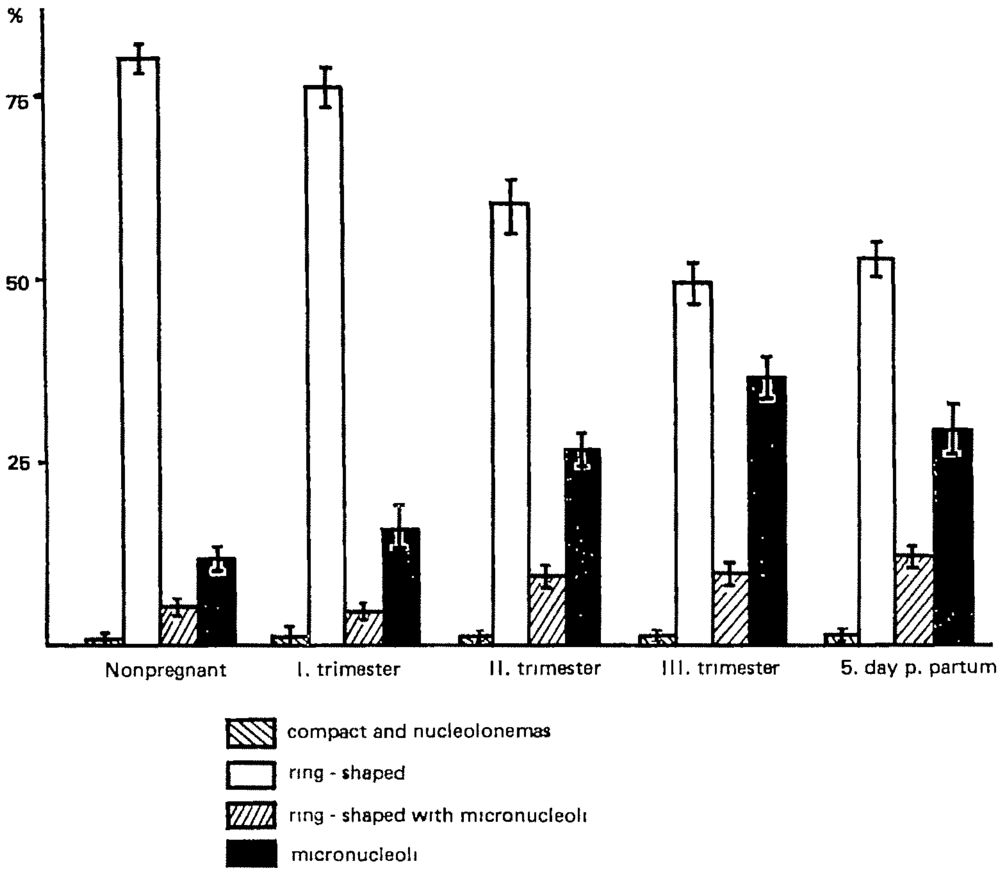

FIG. 4

The appearance of nucleoli in non-pregnant women and in women at various stages of pregnancy. The small vertical lines indicate 95 per cent confidence limits.

There was no relationship revealed between the percentages of micronucleoli and parity or age.

## DISCUSSION

Morphological changes in lymphocytes after stimulation with antigens (especially PHA) are considered as an activation which is described by many authors as lymphoblastic reaction. Comparatively few authors have studied lymphocytic activation on the basis of nucleolar changes. An increased number of lymphocytes with compact nucleoli or with nucleolonemas has been observed in experimental infections in animals (Pekárek *et al.*, 1972), after PHA (Likovský *et al.*, 1973) and after skin graft implantation (Korčáková, 1973). This increase was at the expense of lymphocytes with ring-shaped nucleoli.

Potměšil and Smetana (1969) regarded ring-shaped nucleoli in human lymphocytes as evidence of their stimulation and micronucleoli as indicative of damage to lymphocytes with irreversible inhibition of RNA synthesis. In infectious diseases a greater number of active lymphocytes may be seen, but the number of lymphocytes with micronucleoli remains the same (Šrámková, 1973).

We have found no statistically significant increase of activated lymphocytes in pregnancy. On the other hand a significant increase of lymphocytes with micronucleoli alone as well as in combination with ring-shaped nucleoli was observed. This shift was associated with a decrease of ring-shaped nucleoli. As there were less lymphocytes with micronucleoli found on the fifth day after delivery and more lymphocytes with ring-shaped nucleoli, it seems that the change of ring-shaped nucleoli into micronucleoli was reversible. It is also of importance that the absolute number of lymphocytes did not change during pregnancy. An increase of leucocytes but no significant change in the number of lymphocytes during pregnancy has been reported (Hytten and Leitch, 1969; Brain, 1972).

Our results indicate a very close relationship between pregnancy and the occurrence of micronucleoli in lymphocytes. We suggest that the micronucleoli reflect a nonspecific reaction to pregnancy for we find them in very small numbers in non-pregnant women. The higher number of micronucleoli might be significant of the inhibition of RNA synthesis in lymphocytes. This view is supported by the decreased reactivity to PHA (estimated by the use of morphological methods) and by the lowered antibody response to some infectious diseases during pregnancy (Stickl, 1972). On the other hand, decreased reactivity was not found by labelling methods (incorporation of $^3$H-thymidine), and strong antibody reaction to specific stimuli is very well known in pregnant women (as with rhesus isoimmunization). Our preliminary conclusions are: (1) that pregnancy alone does not "increase" lymphocytes as other antigenic stimuli do; (2) that progressive increase in the incidence of lymphocytes with micronucleoli during pregnancy and the fall after delivery indicate a close relationship between pregnancy and micronucleoli, and (3) that it is not known whether the increase in micronucleoli is due to an irreversible inhibition of RNA synthesis or to a fetal defence mechanism.

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# STUDIES ON COAGULATION AND FIBRINOLYSIS IN BLOOD FROM PUERPERAL WOMEN WITH AND WITHOUT OESTROGEN TREATMENT

BY

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## Summary

Coagulation and fibrinolytic studies were made on 25 women who had a normal puerperium and breast fed their babies and on 32 otherwise healthy women who had lactation suppressed by diethylstilboestrol. In the untreated patients the fibrinogen, Factors VIII and IX, platelet count, and antifibrinolytic activity increased, and the levels of Factors II, VII, and X and of plasminogen and plasminogen proactivator decreased. The incidence of cold activation of Factor VII decreased slowly from the 5th postpartum day onwards. The concentration of antithrombin III which was depressed in pregnancy, increased in the puerperium and was the only observed change that counteracted an increased coagulation potential. Oestrogen treatment produced further increases of Factors II, VII, and X, platelet count, and the cold activation of Factor VII while it decreased the concentration of antithrombin III. These changes favoured coagulation but were possibly to some degree counterbalanced by an increased concentration of plasminogen and decreased antifibrinolytic activity.

THE tendency to thromboembolic disease in the puerperium has not yet been fully explained, but we know that it is probably increased if lactation is suppressed by oestrogens (Daniel *et al.*, 1967; Jeffcoate, 1968). We therefore investigated the effect of diethylstilboestrol on changes that occur during the puerperium in the blood components which have a presumptive relation to thrombogenesis.

## METHODS

Blood was obtained from two groups of women:

1. A group of 25 women who had a normal puerperium and who were breast feeding their babies.

2. A group of 32 healthy puerperal women who had their lactation suppressed by diethylstilboestrol given in doses of 15 mg. a day for three

days, then 10 mg. a day for two days, and finally 5 mg. a day for one day. In 16 of the women breast engorgement necessitated a second course of treatment.

Plasma was prepared by the method described by Gjønnæss (1972*a*). The thrombotest and normotest systems (Owren, 1959 and 1969), both mainly reflect the concentrations of Factors VII and X, but thrombotest is more sensitive to possible activators and inhibitors than normotest. In some cases extraordinarily high thrombotest values were recorded and in the further calculations these were taken to represent 200 per cent activity. The normotest values tended to be within the normal physiological range. Table I records the other measurements that were made and the names of workers whose methods were used. From each woman blood was taken before delivery and then 8 to 12 times during the first four weeks of the puerperium. Each subject served as her own control. The differences within the groups were evaluated with Student's "t" test for paired comparison. The comparison between the untreated and the oestrogen-treated series was evaluated by a two-sample rank test (Wilcoxon-White), or, when the sample sizes exceeded 20, by an ordinary Student's "t" test. The differences between the incidence of cold activation of Factor VII in the two series were evaluated by a Double Bernoulli test series (Sverdrup, 1964).

TABLE I

*Coagulation parameters measured and methods employed*

| Measurement | Method |
|---|---|
| Cold Activation of Factor VII | Gjønnæss (1972*a*) |
| Cephalin time | Proctor and Rapaport (1961) |
| Factors VIII and IX | Schiffman *et al.* (1963) |
| Fibrinogen | Godal (1961) |
| Soluble fibrin, ethanol test | Godal and Abildgaard (1966) |
| Platelet count | Bøyum *et al.* (1971) |
| Plasminogen/plasmin, caseinolysis with NEU | Jacobsen (1968*a*) |
| Proteolytic capacity | Jacobsen (1968*b*) |
| Plasminogen proactivator | Blix (1962) |
| Antiplasmin activity | Jacobsen (1968*a*) |
| $\alpha_1$-antitrypsin, $\alpha_2$-macroglobulin and antithrombin III | Mancini *et al.* (1965) |

## RESULTS

None of the patients developed signs of thromboembolic disease.

### *Plasma coagulation*

*Extrinsic system.* Both the thrombotest and the normotest levels were increased in pregnancy. After delivery the untreated women reached normal thrombotest and normotest levels in four to five days and two weeks respectively (Fig. 1). The effect of oestrogen (the difference between treated and untreated patients) on the thrombotest values ($p<0{\cdot}01$) could be an effect of activation, while the effect on the normotest probably indicates increased concentration of the coagulation factors ($p<0{\cdot}01$). At four weeks after delivery the average thrombotest level was still significantly higher ($p<0{\cdot}05$) in the women who had two courses of oestrogen than in the untreated patients.

Before delivery, cold activation of Factor VII was observed in the plasmas of 94 per cent of the women (Fig. 1). In the untreated series the incidence decreased from the 5th postpartum day onwards, reaching a normal level in three to four weeks. Oestrogen treatment influenced this incidence so that 11 to 13 days after delivery 100 per cent of the oestrogen-treated women, and only 33 per cent of the untreated, showed cold activation of Factor VII ($p<0{\cdot}05$). The oestrogen-treated patients showed a normal incidence of cold activation of Factor VII at four weeks after delivery.

*Intrinsic system.* The cephalin time, which is a screening test for the intrinsic coagulation system, was significantly shorter in pregnancy than four weeks after delivery ($p<0{\cdot}001$), probably due to increased levels of Factors VIII (about 50 per cent; $p<0{\cdot}001$) and IX (about 24 per cent; $p<0{\cdot}001$) (Fig. 2). A slight shortening of the cephalin time was found in the plasmas of the untreated series four to five days after delivery, possibly corresponding to the increased levels of Factors VIII ($p<0{\cdot}001$) and IX ($p<0{\cdot}001$). Later on, the cephalin time showed a steady increase, in parallel with the reductions in the concentrations of Factors VIII and IX. Normal values were achieved about two weeks after delivery.

At four to eight days after delivery the oestrogen-treated group showed a longer mean

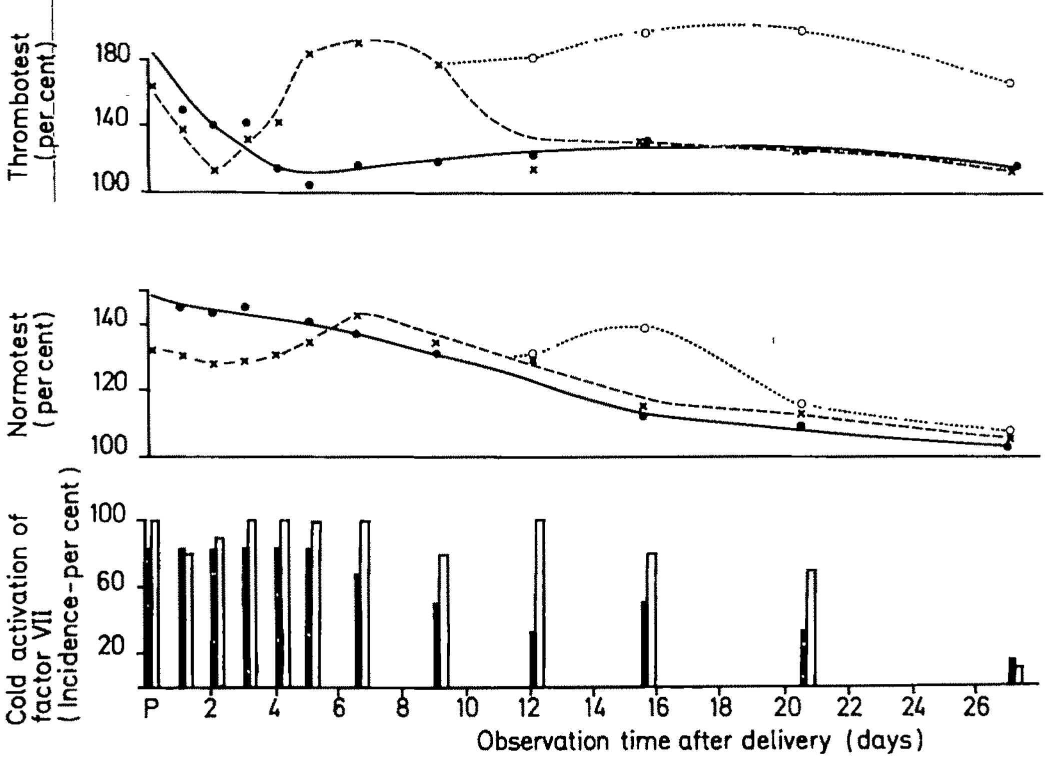

FIG. 1

The two top graphs show changes in the extrinsic coagulation system in untreated —●————●— and oestrogen-treated (1 course of treatment + – – – – – +, 2 courses of treatment - - O - - - - O - -) puerperal women. Each point indicates a mean value. The histogram illustrating cold activation of Factor VII shows the percentage of plasma samples that showed thrombotest times under 20 seconds after 20 hours incubation at 0 °C. The shaded columns represent untreated patients while the open columns represent treated patients. (P = mean values pre-delivery.)

cephalin time than the untreated group ($p<0{\cdot}02$) together with a possible decrease in the Factor VIII and IX levels.

*Plasma fibrinolysis*

The urokinase activated caseinolysis in the presence of the natural plasma proteinase inhibitors, the "proteolytic capacity", was significantly higher in pregnancy than four weeks after delivery ($p<0{\cdot}001$). The proteolytic capacity decreased and became normal in two weeks (Fig. 3).

A similar assay with N-ethylurethane-induced destruction of the inhibitors reflects mainly the plasminogen concentration, and it was significantly higher before delivery than four weeks after ($p<0{\cdot}001$). In the untreated patients the values decreased after delivery, but the decrease was somewhat delayed when compared to that of the proteolytic capacity.

The existence of the streptokinase activated plasminogen proactivator was considered dubious until recently when the proactivator was probably purified (Takada *et al.*, 1972). In pregnancy the levels were about 50 per cent higher than four weeks after delivery ($p<0{\cdot}05$), and in the untreated series the values became normal in two to three weeks. A slight increase on the second and third postpartum days was not statistically significant.

Oestrogen treatment markedly increased the proteolytic capacity so that three weeks after delivery the difference between the patients that had a second course of oestrogens and the

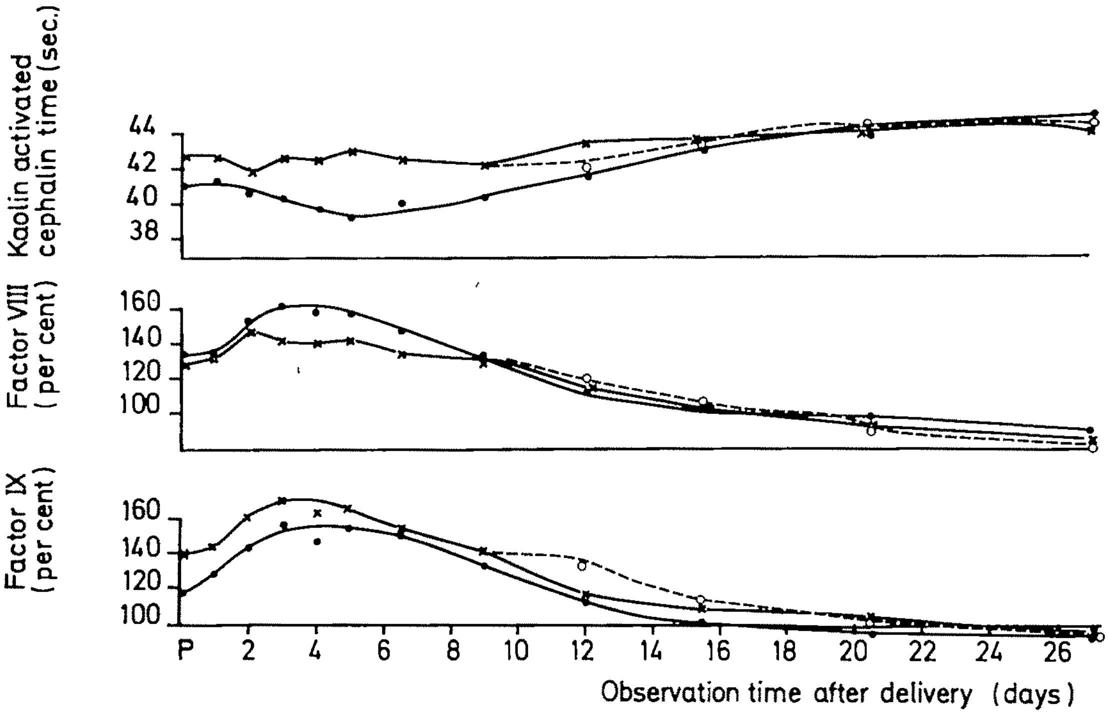

FIG. 2
Changes in the intrinsic coagulation system. (See Figure 1.)

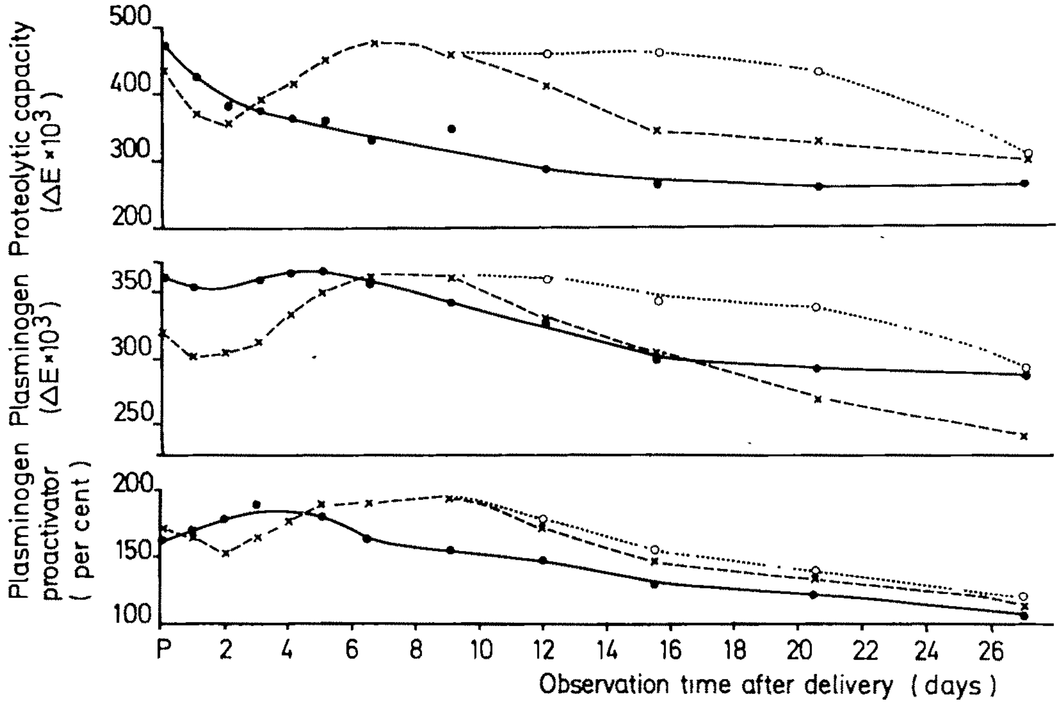

FIG. 3
Changes in the fibrinolytic system. (See Figure 1.)

untreated patients was still highly significant ($p<0{\cdot}001$). This difference was significant also for the plasminogen concentration ($p<0{\cdot}01$) and the proactivator activity ($p<0{\cdot}001$). The difference between the average plasminogen levels of the untreated and the treated patients before delivery was not significant. Four weeks after delivery the difference persisted, possibly as an effect of small groups and variation between individuals.

### *Proteinase inhibitors*

In the untreated women the plasmin inhibiting activity in plasma was about 30 per cent lower before delivery than four weeks after ($p<0{\cdot}05$), and the antepartum values were all taken to represent 100 per cent inhibitor activity. In pregnancy the concentration of $\alpha_1$-antitrypsin was about 75 per cent higher than four weeks after delivery ($p<0{\cdot}001$), while the concentration of $\alpha_2$-macroglobulin was similar in pregnancy and the puerperium (Fig. 4).

The postpartum increase in antiplasmin activity was prevented by oestrogen treatment, the difference between the two series being significant ($p<0{\cdot}05$) up to four weeks after delivery. Oestrogen treatment increased the concentration of $\alpha_1$-antitrypsin so that the difference between the averages for the untreated and the oestrogen-treated patients was also significant four weeks after delivery (two courses of treatment: $p<0{\cdot}001$; one course of treatment: $p<0{\cdot}005$). The concentration of $\alpha_2$-macroglobulin was not significantly influenced by oestrogen treatment.

The mean plasma concentration of anti-

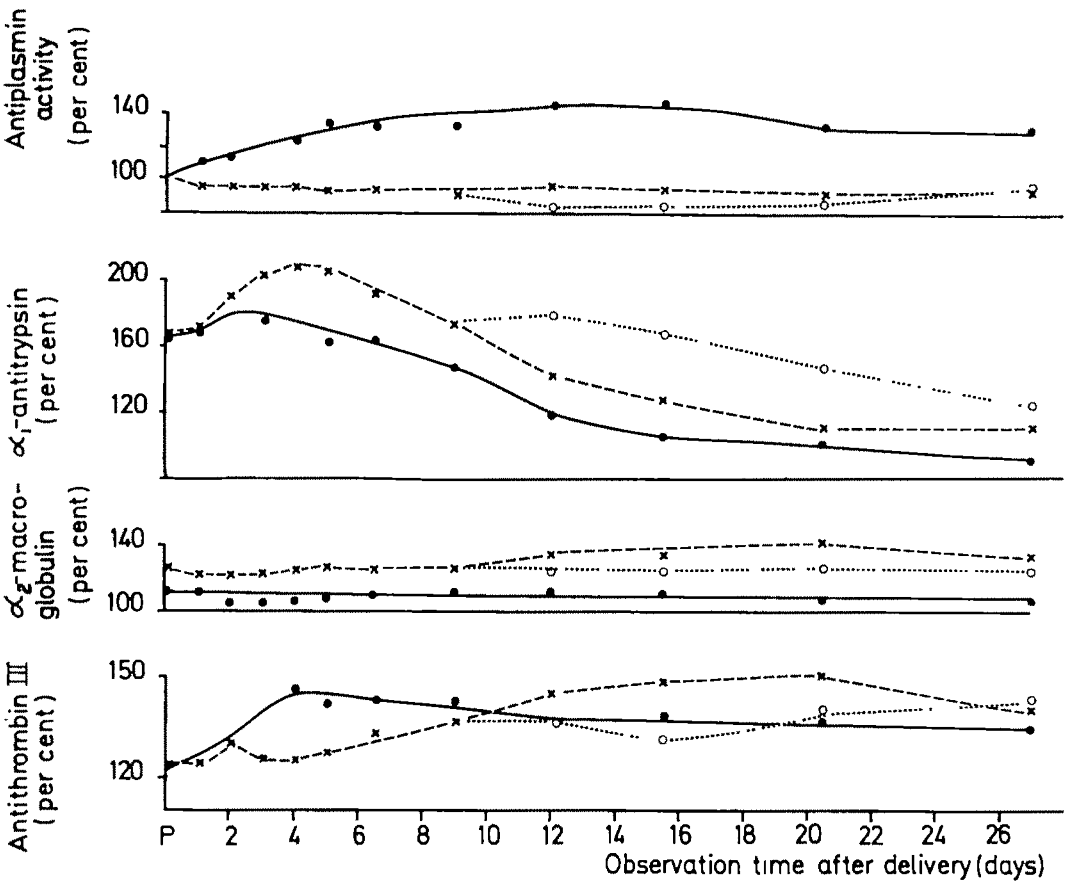

FIG. 4
Changes in the proteinase inhibitors. (See Figure 1.)

thrombin III was 18 per cent lower in pregnancy than at four weeks after delivery ($p<0\cdot05$), but in untreated subjects the values already became normal on the second postpartum day. Oestrogen treatment reduced the concentration of antithrombin III. Instead of increased values four to five days after delivery when compared to the four weeks postpartum value, decreased values were recorded in 10 of 18 subjects, and the difference between the untreated and the oestrogen-treated series was statistically significant ($p<0\cdot05$).

### *Fibrinogen, haematocrit and platelet count*

In spite of the high fibrinogen concentration in pregnancy (about 460 mg./100 ml.) a further increase ($p<0\cdot01$) occurred during the first postpartum week (Fig. 5). The fibrinogen concentration then levelled off and reached normal values three to four weeks after delivery. The influence of oestrogen treatment was not statistically significant. Soluble fibrin was looked for, but the ethanol test was always negative.

In untreated puerperal women no significant change in the average haematocrit value could be demonstrated. Oestrogen treatment induced a minor decrease ($p<0\cdot05$) which was presumably too small to influence the assays in this report by the variation of plasma dilution and calcium chelation on the addition of citrate.

The platelet count increased significantly ($p<0\cdot001$) after delivery, and then decreased slowly. Four weeks after delivery it was still higher than before delivery ($p<0\cdot05$). Oestrogen treatment increased the platelet count, so that 11 to 13 days after delivery there was a statistically significant difference between the treated and the untreated patients ($p<0\cdot01$).

## DISCUSSION

In the untreated puerperal patients an increased coagulation potential was indicated by the increases in Factors VIII and IX, fibrinogen, and platelet count, in agreement with the report of Ygge (1969). Hypercoagulability, however, is probably not related to the total quantity only of a specific clotting factor in the circulation but to whether it is circulating in activated form

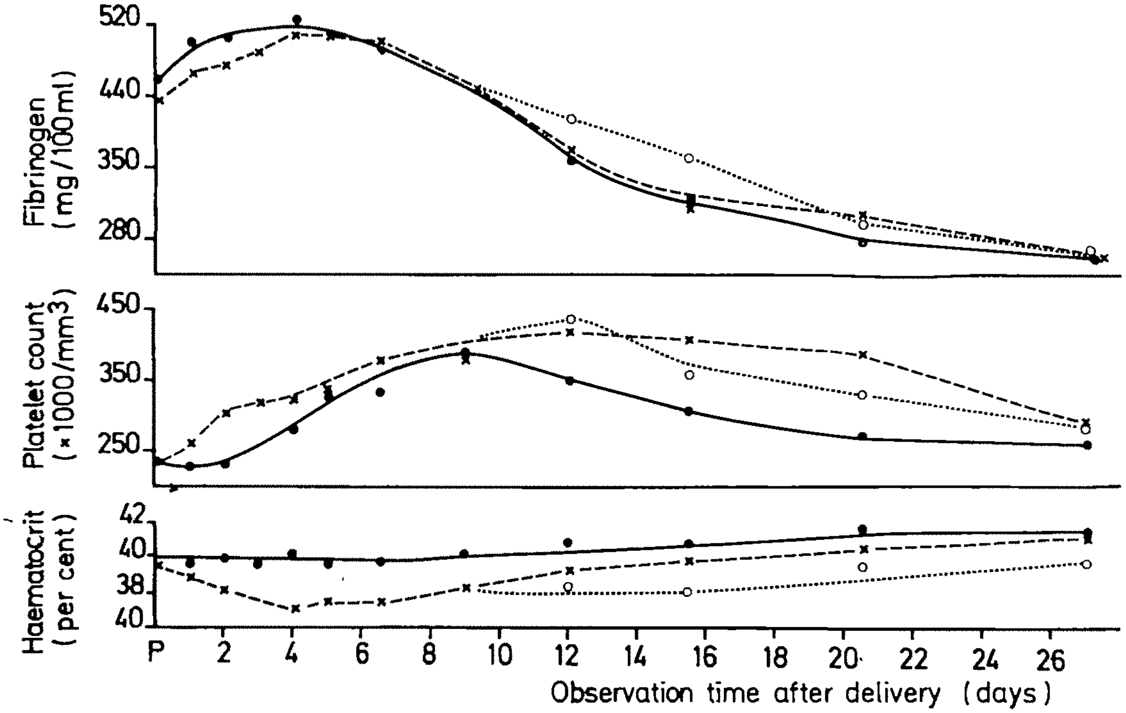

FIG. 5

Changes in fibrinogen concentration, platelet count and haematocrit. (See Figure 1.)

(Wessler, 1971). Accordingly, the cold activation of Factor VII might be of importance. This test, which reveals the effect of plasma kallikrein upon Factor VII (Gjønnæss, 1972*b*) is performed after 20 hours incubation at 0 °C. to reduce the effect of the plasma inhibitors of kallikrein (Laake *et al.*, 1973). Theoretically, any *in vivo* reduction of the kallikrein inhibiting activity might trigger a similar Factor VII activation. The incidence of cold activation was high before delivery (Fig. 1; Gjønnæss, 1973), and it became normal after the thrombotest level, because the two observations reflect different phenomena.

The main effects of oestrogen treatment were an increased platelet count, increased concentration of the factors of the extrinsic coagulation system, an increased incidence of cold activation of Factor VII, and a decreased concentration of antithrombin III. The oestrogen-induced changes in these factors thus seem to favour coagulation.

In the fibrinolytic system the effect of oestrogen treatment was apparently the reverse. In agreement with other reports (Menon *et al.*, 1970; Ygge, 1969), the plasminogen concentration increased and the antifibrinolytic activity decreased, resulting in increased proteolytic capacity (Fig. 3). The fibrinolytic system is, however, complex and concerns activators and inhibitors that are not at the time fully identified. For instance, the Malmö group has demonstrated low fibrinolytic activity of the venous wall during pregnancy (Åstedt *et al.*, 1970) and also as an effect of oestrogen treatment (Åstedt, 1971). Further, the fibrinolytic system may reveal effects that are thrombogenic as well as thrombolytic.

It is concluded, that oestrogen treatment in the puerperium, as well as the puerperium *per se*, cause gross changes in the blood. The *in vivo* effect of such changes is extremely difficult to evaluate because of the complexity of the factors concerned in thrombogenesis. Apparently, the "dynamic equilibrium" (Astrup, 1956) between coagulation and fibrinolysis is maintained on another level.

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# THE RELATIONSHIP OF A "SHORT PERIOD" TO CONCEPTION

BY

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## Summary

The case records were examined of 918 patients delivered in 1963 at a London hospital. Comparisons were made between 52 patients who had a "short period" following the presumed date of conception and 155 randomly selected control patients. Patients who had a "short period" showed a significantly higher rate for the induction of labour ($p<0 \cdot 025$) and a lower infant birthweight ($0 \cdot 1<p<0 \cdot 2$). Calculation of the menstruation-delivery interval using the "short period" rather than the last full period, gave a closer approximation to the duration of pregnancy found in the random control series of 155 patients.

It is commonly taught that after fertilization, a woman may have one or even two "short periods" at the time when menstruation would normally have taken place (Gibberd, 1965; Browne and Dixon, 1970; Brown, 1971). Small episodes of painless bleeding in the first eight to 10 weeks of pregnancy are often dismissed in this way and thus in assessing the expected date of delivery, the last normal menstrual period is accepted for the calculation. It is suggested here that in a significant number of cases, the "short period" comes before rather than after conception and that labour may be induced for post-maturity when, in fact, the fault lies with the calculated gestational maturity. This view is supported by the work of Beischer *et al.* (1969*a*) who found that when bleeding occurred early in a pregnancy which subsequently continued beyond 28 weeks gestation, there was a highly significant correlation with prolonged pregnancy ($p<0 \cdot 005$).

## Methods

This analysis is based on the 918 available records from deliveries in 1963 at a teaching hospital. In those cases in which a "short period" (two or more days below the patient's usual menstrual length) was definitely recorded, the estimated date of delivery was calculated in two ways assuming conception predating or post-dating the "short period".

A random series was obtained by selecting cases in sequence using a table of random numbers. Patients with multiple pregnancy, fetal abnormality, intrauterine fetal death and serious maternal disease were excluded, as were all patients delivered by Caesarean section and those in whom there was significant doubt about menstrual dates, no menstrual history or gross menstrual irregularity before pregnancy. After these exclusions there remained a random control group of 155 patients and 52 patients in the "short period" group. In each case the birthweight of the infant was recorded as well as the mode of onset of labour.

## Results

The mean menstruation-delivery interval for the control series was 282·2 (SD±18·8) days. Using the "short period" as a basis for calculation, the interval in days was 271·8 (SD±22·7) and using the last full period 302·1 (SD±21·6) (see Fig. 1). The distribution of patients in the two histograms is not identical because if two "short periods" were recorded, both were taken

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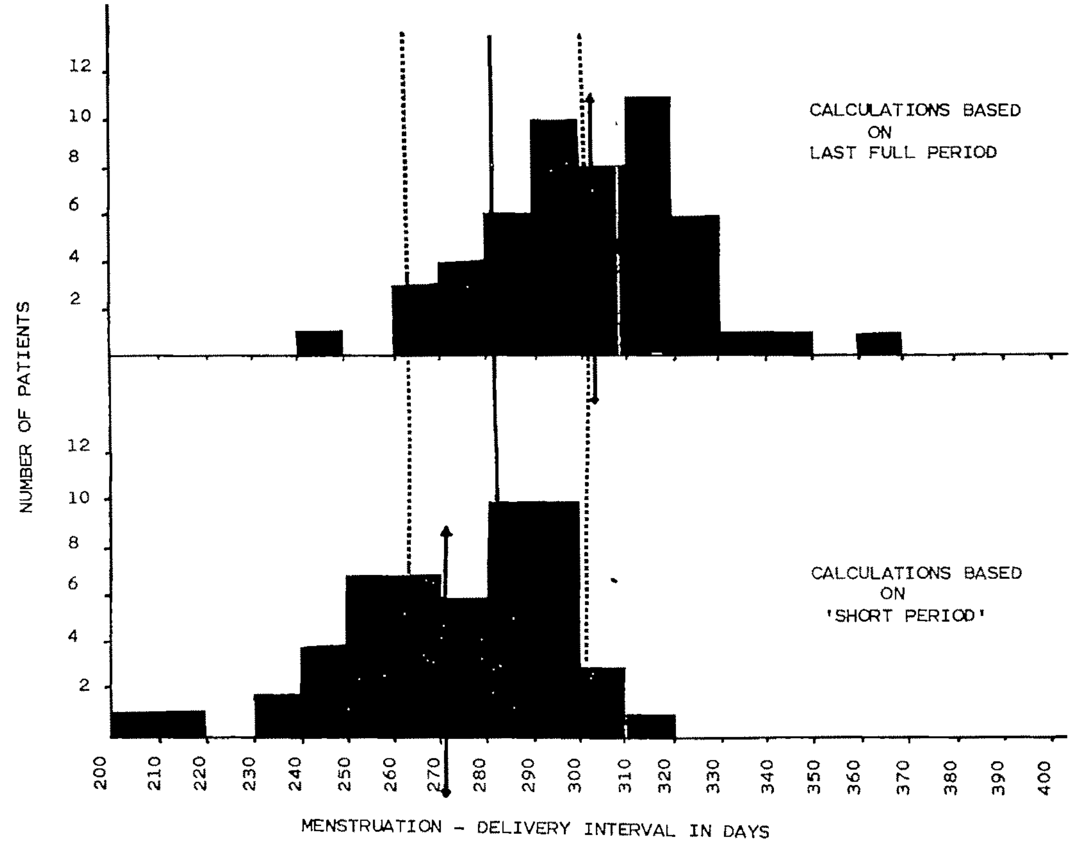

Fig. 1

The menstruation-delivery interval using the two alternative calculations is shown as a histogram divided into groups of 10 days. The central solid line and the broken lines on either side show the mean and the standard deviations for the control group. The mean for each separate calculation is shown by an arrow tipped line.

into account when calculating the menstruation-delivery interval.

The mean birthweight for the control series was 3·31 kg. (SD±0·59 kg.) and for the patients who had a "short period" 3·15 kg. (SD±0·59 kg.). The number of surgically induced labours was 26 (15·3 per cent) in the control patients and 17 (32·7 per cent) in the patients who had a "short period" (Fig. 2).

## Discussion

### *Menstruation-delivery interval*

The menstruation-delivery interval is the most convenient reference point for clinical use. Treloar *et al.* (1967) found that ovulation occurred over a range of 1 to 34 days after the last menstrual period and that in 70 per cent of pregnancies, the menstruation-delivery interval was 280±10 days.

It has been argued that menstrual data without written records are almost valueless as scientific evidence (Gunn *et al.*, 1937; Arey, 1939). However, the menstruation-delivery interval in the control patients correlates well with larger statistical analyses (Hotelling and Hotelling, 1932; Hammes and Treloar, 1970).

Figure 1 shows that there is a significant disparity in the menstruation-delivery interval which is much more marked using the last full period ($p<0.000001$) than the short period ($p<0.01$). The deviation of both groups from the control series clearly shows that the "short period" series is a heterogeneous group and that some cases do represent true bleeding in early

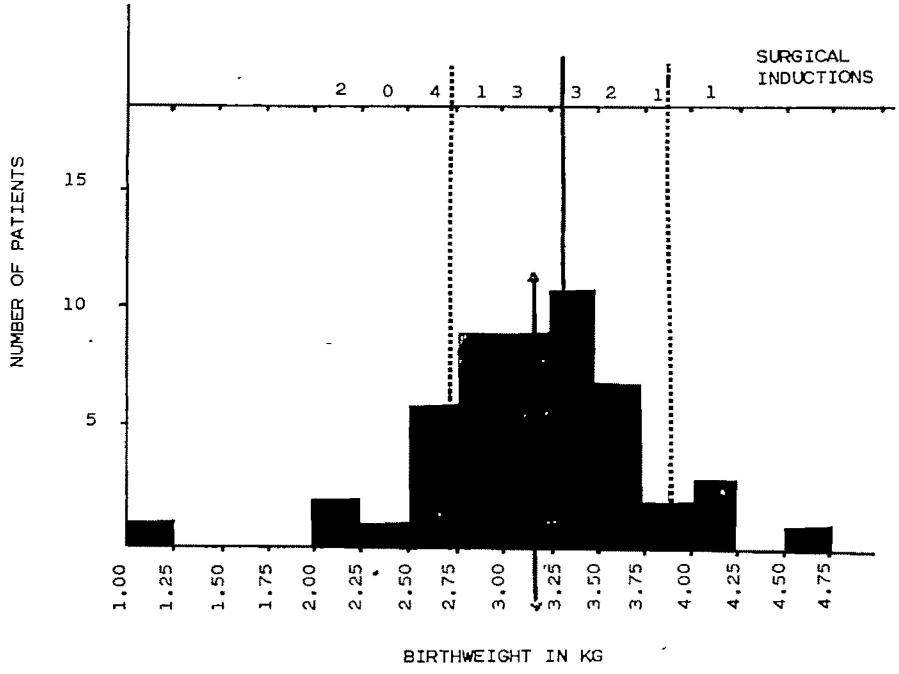

FIG. 2

Birthweights are shown as a histogram in 250 g. groups. The central solid line and the broken lines on either side represent the mean and standard deviations for the control series. The mean for the short period group is shown as an arrow tipped line. The number of surgical inductions in each 250 g. group is shown on the horizontal line above the histogram.

pregnancy. This may represent implantation bleeding, which is known to occur in the Macacus monkey (Hartman, 1930), change to placental hormone dominance (Johansson, 1969; Biggs, 1972) or simply bleeding from the choriodecidual space. However, Figure 1 shows that the calculation using the "short period" approaches the control group more closely and indeed the mean value using the last full period lies outside the standard deviation of the control series.

### *Birthweight*

The correlation between gestational age and birthweight is only moderate (Battaglia, 1970; Yerushalmy, 1970); relevant variable factors include race, diet and fetoplacental insufficiency. The mean birthweight in this study correlates well with more recent statistics from this hospital and with a large analysis of deliveries from the area (Barron and Vessey, 1966). The mean birthweight for the "short period" group is suggestively lower than that in the control series and does not correspond with post-maturity which has been shown to be associated with increased mean birthweight (Beischer *et al.*, 1969*b*).

### *Surgical induction*

There was a high percentage (32·7 per cent) of surgical induction of labour in the "short period" group when compared with the control series (15·3 per cent) and this level of induction is of more significance than it would be today when surgical induction is undertaken much more readily. It also occurred despite the constant re-evaluation of gestation always made during the antenatal period, from observations of first fetal movements, uterine growth and even assessment of the cervix preoperatively. Six of the 10 babies whose birthweight was below the standard deviation had labour induced and two of them weighed less than 2·5 kg. (Fig. 2).

The year 1963 was chosen because since then three major developments in obstetrics have complicated the calculation of the menstruation-delivery interval in "short period" cases. Widespread use of the contraceptive pill allows many patients little time to re-establish normal menstruation before conception. The increasing accuracy of mechanical and biochemical aids in assessment of fetal gestation, particularly ultrasound, has meant less effort is often spent in defining and recording exactly the immediate menstrual history. Finally, the concept of a small-for-dates baby has emerged and separation of this group would bias the results for both surgical induction of labour and for fetal weight in relation to maturity.

The decision to exclude all patients who had a Caesarean section was reached with some reluctance since patients in whom induction fails to achieve good labour are often the most premature. It was not possible, however, always to isolate a single specific indication for Caesarean section and inclusion of cases with two or more factors present would again bias the series. Hotelling and Hotelling (1932) in their analysis found a racial bias in gestation lengths which only disappeared after several factors, including Caesarean section, stillbirth and venereal disease had been excluded.

There are few published studies on the length and variation of periods in an individual woman but Geist (1930) found that in short (1 to 3 days) and normal (4 to 5 days) periods the deviation was usually less than 24 hours. Quite why a short period should precede conception is unknown but perhaps it represents a change in hormone climate related to increased infertility.

Postmaturity is associated with increased fetal hazard but this paper suggests that a significant number of infants are exposed to the greater risk of prematurity because the calculated date of delivery has been based on a false assumption.

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# CARBOHYDRATE METABOLISM IN THE MENSTRUAL CYCLE

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## Summary

Blood sugar, serum insulin and growth hormone levels in response to 100 g. of oral glucose were studied under carefully controlled conditions on days 9, 18 and 27 of the menstrual cycle in 20 healthy women aged 18 to 20 years. The results indicate that blood sugar and serum insulin levels in response to oral glucose loading do not alter significantly in the menstrual cycle. Seven of the women had repeat studies on the same days three cycles later. No significant change in blood sugar or serum insulin levels occurred between cycles. The fasting growth hormone level was highest at the time of impending menstruation in 11 subjects (55 per cent) but the difference in mean values for the group as a whole was not statistically significant. It is concluded that the changes in endogenous hormone levels in a menstrual cycle do not produce significant alterations in carbohydrate metabolism in normal women in this age group. The reported divergence of blood glucose and insulin levels after oral glucose in the menstrual cycle may reflect the interaction of other factors such as age and parity on glucose tolerance. It is suggested that investigations into the metabolic effects of oral contraceptive steroids on these parameters should be performed in nulliparous women in a similar age range.

IT is well known that difficulty may arise in the control of insulin requiring diabetics just prior to or during menstruation, and recently there have been many reports indicating that altered glucose tolerance and insulin response may occur in pregnancy and in women receiving oral contraceptive steroids. Hormonal effects, in particular from oestrogen and progesterone, and in the brittle diabetic a possible additional disturbance in growth hormone, have been invoked in an attempt to explain these observations, but only on occasions has reference been made to the possible alterations in blood glucose and insulin levels in response to the natural changing hormonal background of the menstrual cycle in normal women.

The oral glucose tolerance test (OGTT) is the most frequently employed method of assessing glucose homeostasis. It is both more acceptable to the patient and more physiological. The purpose of this report is to present data on blood sugar, serum insulin and growth hormone levels in response to *oral* glucose, in normal nulliparous women of similar age, tested at the same times within a menstrual cycle and in different cycles.

## METHODS

The subjects were 20 normal female medical students aged between 18 and 20 years (mean 19·05 years). The weight of each subject was within 5 per cent of average normal weight (range 41·4 to 76·2 kg.; mean 56·8 kg.). The menarche ranged from 9 to 16 years (mean

13·3 years), and each individual had a normal menstrual cycle (range 27 to 30 days; mean 28·2 days). Enquiry was made to ensure a normal dietary history and exclude any family history of diabetes mellitus. No drugs were taken during the period of the study and smoking was not allowed before or during the tests. No attempt was made to ascertain the time of ovulation.

A 100 g. OGTT was carried out on days 9 (8 to 10), 18 (17 to 19) and 27 (26 to 28), the first day of the cycle corresponding to the first day of menstrual bleeding. Thus each subject had three equally spaced tests occurring in the secretory and proliferative phases and at the time of impending menstruation. Seven subjects had repeat studies on the same days three cycles later.

Each subject was fasted from midnight and on arrival was seated in a quiet room for thirty minutes prior to commencing the test. The fasting sample was taken between 0900 and 1000 hours to eliminate inter-subject variability due to changes in circadian rhythm. The glucose in 400 ml. of water (at about 8 °C.) was sipped steadily over four minutes, and venous blood samples were collected at 30, 90 and 120 minutes from the beginning of the ingestion of the glucose.

Blood sugar was estimated within four hours using a Technicon autoanalyzer (Ferricyanide method). This does not measure glucose alone, hence the use of the term "blood sugar". Serum was separated thirty minutes after collection and stored at —20 °C. Later, all the serum samples from the same patient were analyzed in duplicate in the same assay. Radioimmunoassay was carried out by the method of Hales and Randle (1963) and growth hormone (GH) according to Schalch and Parker (1964). The results for GH were expressed in terms of the first international reference preparation (IRP) 66/217.

The data were subjected to a variety of statistical tests in order that explainable and chance variations in the measurements could be separated as far as possible, and to identify significant sources of variation. Three factor analyses of variance were performed on the individual blood sugar values and on the logarithms of the individual insulin levels within a cycle, and, for blood sugar, on the differences between corresponding values in the two cycles. In each case a mixed linear model was adopted regarding the factor "subjects" as a random sample and the factors "days" and "times" as fixed. The area under the curve (0 to 120 minutes) for both glucose-time and insulin-time was calculated for each individual using the formula $a+2b+2c+2d+e$, where a is the fasting value and b, c, d and e are the subsequent half-hourly measurements. Two and three factor analyses of variance were performed on these values, based on mixed linear models similar to those described above.

## RESULTS

### *Blood sugar*

No fasting or two hour blood sugar value was greater than 105 mg./100 ml. and the highest peak blood sugar for any individual was 137 mg./100 ml., indicating normal glucose tolerance. The individual values (Table I) were approximately normally distributed and the means and standard deviations of the values for the three tests within one cycle, together with the mean blood sugar-time areas, are shown in Table II. The analyses showed that, within the scope of the model, there were no significant systematic differences in blood sugar levels on the three days of the cycle although it was apparent that the subjects showed significantly inconsistent variations between the days. There were highly significant differences between the subjects albeit within the range of normal glucose tolerance. No significant differences in glucose-time areas were shown to exist between days although there were significant differences between subjects.

### *Serum insulin*

The coefficient of inter-assay variation was 4·3 per cent. The individual insulin levels (Table I) show a characteristic positively skewed distribution at each time after oral glucose. Conversion to log values produced approximate normality of distribution. The means and standard deviations of the raw values for the three tests within one cycle, together with the mean insulin-time areas, are shown in Table II. The results of the analyses of log insulin and insulin-time areas are consistent with those found for blood sugar and similar conclusions may be drawn. Specifically, no systematic

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*Individual blood sugar and serum insulin levels, plus calculated blood sugar and ins*

| Subject | Day 9 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Time (minutes) | | | | | Area | | Time (min | | |
| | 0 | 30 | 60 | 90 | 120 | GTT | Insulin | 0 | 30 | 60 |
| 1 | 73 | 108 | 71 | 70 | 70 | 641 | | 83 | 113 | 75 |
| | 7·9 | 28·5 | 21·5 | 17·4 | 15·3 | | 158 | 7·3 | 62·5 | 26· |
| 2 | 73 | 85 | 50 | 41 | 48 | 473 | | 80 | 122 | 80 |
| | 6·7 | 60·2 | 55·9 | 30·3 | 21·9 | | 321 | 6·6 | 53·9 | 45· |
| 3 | 77 | 114 | 91 | 65 | 68 | 685 | | 70 | 99 | 83 |
| | 13·6 | 12·2 | 42·5 | 79·8 | 69·7 | | 352 | 9·5 | 60 1 | 22· |
| 4 | 74 | 82 | 68 | 50 | 58 | 532 | | 65 | 106 | 90 |
| | 5·9 | 66·0 | 46·0 | 20·8 | 36·6 | | 308 | 5·8 | 61·3 | 77· |
| 5 | 85 | 115 | 98 | 85 | 83 | 764 | | 74 | 103 | 12 |
| | 6·4 | 33·3 | 53·4 | 32·8 | 42·5 | | 288 | 7·6 | 21·9 | 52· |
| 6 | 72 | 106 | 73 | 80 | 85 | 675 | | 87 | 89 | 73 |
| | 15·1 | 93·9 | 70·4 | 72·1 | 72·1 | | 560 | 8·1 | 73·9 | 50· |
| 7 | 83 | 122 | 81 | 78 | 74 | 719 | | 81 | 125 | 95 |
| | 8·2 | 65·3 | 80·7 | 65·8 | 62·7 | | 489 | 4·1 | 43·7 | 44· |
| 8 | 76 | 94 | 74 | 79 | 81 | 651 | | 70 | 117 | 87 |
| | 11·7 | 76·2 | 63·4 | 52·9 | 62·0 | | 459 | 10·0 | 90·1 | 91· |
| 9 | 82 | 96 | 77 | 62 | 96 | 648 | | 78 | 108 | 103 |
| | 3·9 | 78·7 | 99·5 | 28·0 | 104·5 | | 521 | 3·7 | 72·2 | 94· |
| 10 | 75 | 106 | 61 | 51 | 56 | 567 | | 71 | 84 | 70 |
| | 3·4 | 56·8 | 50·0 | 37·3 | 18·8 | | 310 | 3·3 | 20·7 | 26· |
| 11 | 72 | 94 | 64 | 59 | 74 | 580 | | 77 | 109 | 68 |
| | 2·9 | 32·3 | 46·8 | 50·3 | 23 8 | | 285 | 5·0 | 49·9 | 36· |
| 12 | 69 | 132 | 102 | 91 | 68 | 787 | | 70 | 94 | 7 |
| | 5·4 | 82·7 | 85·3 | 79·5 | 47·3 | | 548 | 7·3 | 72·3 | 80· |
| 13 | 75 | 89 | 94 | 53 | 62 | 609 | | 70 | 109 | 119 |
| | 6·0 | 50·0 | 68·0 | 40·0 | 50·7 | | 373 | 6·8 | 60·7 | 84· |
| 14 | 78 | 114 | 96 | 84 | 74 | 740 | | 74 | 123 | 104 |
| | 12·2 | 96·0 | 104·3 | 87·5 | 94·1 | | 682 | 10·8 | 74·1 | 62· |
| 15 | 71 | 86 | 81 | 79 | 55 | 618 | | 74 | 98 | 100 |
| | 6·5 | 34·4 | 32·1 | 36·1 | 13·5 | | 225 | 5·0 | 26·8 | 50· |
| 16 | 61 | 100 | 68 | 83 | 75 | 638 | | 76 | 84 | 82 |
| | 13·6 | 53·3 | 24·0 | 42·6 | 58·3 | | 312 | 6·3 | 67·1 | 37· |
| 17 | 74 | 102 | 82 | 80 | 78 | 680 | | 64 | 72 | 81 |
| | 5·8 | 26·0 | 59·2 | 68·8 | 54·7 | | 368 | 6·4 | 64·3 | 51· |
| 18 | 75 | 85 | 51 | 37 | 54 | 475 | | 78 | 113 | 77 |
| | 7·2 | 78·0 | 61·9 | 34·4 | 47·7 | | 403 | 7·4 | 59·4 | 55· |
| 19 | 82 | 97 | 82 | 69 | 63 | 641 | | 78 | 106 | 98 |
| | 6·0 | 27·1 | 29·5 | 50·0 | 36·6 | | 256 | 6·8 | 44·8 | 78· |
| 20 | 80 | 101 | 76 | 60 | 60 | 614 | | 74 | 130 | 110 |
| | 7·3 | 57·6 | 84·6 | 27·7 | 28·8 | | 386 | 6·5 | 129·0 | 139· |

*rea values for 20 normal women on Days 9, 18, and 27 of a menstrual cycle*

| | | | | Day 27 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Area | | Time (minutes) | | | | | Area | |
| 90 | 120 | GTT | Insulin | 0 | 30 | 60 | 90 | 120 | GTT | Insulin |
| 75 | 71 | 680 | | 71 | 81 | 61 | 65 | 65 | 550 | |
| 3 9 | 19·3 | | 252 | 6·3 | 28·9 | 15·9 | 8·3 | 5·2 | | 118 |
| 69 | 69 | 691 | | 84 | 102 | 67 | 53 | 68 | 596 | |
| 8·2 | 22·7 | | 285 | 5·3 | 43·3 | 30·2 | 22·4 | 26·3 | | 223 |
| 51 | 77 | 633 | | 74 | 107 | 87 | 63 | 72 | 660 | |
| 2·2 | 55·7 | | 295 | 9·0 | 91·6 | 53·3 | 72·5 | 82·1 | | 526 |
| 52 | 59 | 640 | | 83 | 101 | 81 | 75 | 75 | 672 | |
| 1·3 | 48·5 | | 453 | 5·5 | 72·1 | 64·8 | 57·9 | 46·0 | | 441 |
| 79 | 91 | 771 | | 80 | 120 | 96 | 92 | 84 | 780 | |
| ·9 | 39·5 | | 287 | 4·1 | 27·1 | 69·4 | 27·4 | 14 3 | | 266 |
| 54 | 73 | 592 | | 65 | 55 | 55 | 66 | 51 | 468 | |
| 8·2 | 27·9 | | 341 | 10·3 | 66·5 | 36·6 | 60·4 | 34·2 | | 371 |
| 9 | 105 | 844 | | 89 | 136 | 119 | 91 | 89 | 870 | |
| ·3 | 34·2 | | 368 | 4·4 | 50·7 | 61·9 | 48·2 | 64·0 | | 390 |
| 8 | 68 | 682 | | 89 | 137 | 82 | 72 | 63 | 734 | |
| ·9 | 56 5 | | 555 | 13·2 | 91·9 | 90·9 | 77·5 | 66·5 | | 600 |
| 7 | 79 | 773 | | 84 | 97 | 83 | 90 | 88 | 712 | |
| ·4 | 88·2 | | 656 | 7·1 | 97·9 | 88·1 | 81·2 | 92·8 | | 634 |
| 6 | 60 | 571 | | 71 | 100 | 101 | 83 | 64 | 703 | |
| ·5 | 11·8 | | 183 | 8·9 | 32·0 | 72 8 | 46·9 | 27·7 | | 340 |
| 1 | 65 | 638 | | 78 | 96 | 60 | 65 | 57 | 577 | |
| ·8 | 16·0 | | 291 | 4·6 | 45·2 | 31·0 | 6·2 | 4·9 | | 174 |
| 6 | 56 | 572 | | 73 | 103 | 69 | 52 | 57 | 578 | |
| ·8 | 63·1 | | 518 | 12·1 | 89·7 | 79·2 | 53·7 | 48·5 | | 506 |
| 2 | 78 | 808 | | 77 | 129 | 75 | 83 | 81 | 732 | |
| ·8 | 63·7 | | 530 | 6·1 | 66 1 | 46·3 | 41·1 | 50 7 | | 364 |
| 0 | 97 | 785 | | 77 | 126 | 124 | 100 | 72 | 849 | |
| ·1 | 83·0 | | 477 | 12·0 | 92·8 | 94·4 | 98·2 | 94·7 | | 677 |
| 6 | 62 | 684 | | 62 | 90 | 74 | 60 | 62 | 572 | |
| ·1 | 17·7 | | 249 | 6·0 | 32·8 | 34·2 | 16·2 | 25·1 | | 197 |
| 6 | 76 | 656 | | 74 | 88 | 80 | 82 | 88 | 662 | |
| ·1 | 45·5 | | 342 | 5·8 | 60 8 | 38·5 | 29·8 | 38·2 | | 302 |
| 5 | 73 | 593 | | 75 | 99 | 101 | 87 | 80 | 729 | |
| ·9 | 60·4 | | 362 | 7·0 | 71 6 | 83·1 | 65·1 | 40·0 | | 487 |
| 9 | 67 | 663 | | 68 | 91 | 58 | 70 | 84 | 590 | |
| ·8 | 51 3 | | 410 | 9·3 | 85·4 | 79·1 | 45·1 | 43·1 | | 472 |
| 4 | 64 | 698 | | 75 | 83 | 101 | 59 | 53 | 614 | |
| ·8 | 74·7 | | 451 | 7·4 | 18·5 | 53·5 | 60·1 | 42·2 | | 314 |
| 4 | 69 | 791 | | 80 | 110 | 98 | 94 | 60 | 744 | |
| ·9 | 24·3 | | 725 | 5·2 | 68·5 | 111·3 | 60·6 | 51·5 | | 537 |

TABLE II

*Mean blood sugar, serum insulin levels and values for area under the curve, for 20 normal women on Days 9, 18 and 27 of a menstrual cycle (see Table I)*

| | | Time (minutes) | | | | | Area±SD |
|---|---|---|---|---|---|---|---|
| | | 0 | 30 | 60 | 90 | 120 | |
| Mean blood sugar ±SD | Day 9 | 75·3<br>±5·5 | 101·4<br>±13·4 | 77·0<br>±14·6 | 67·8<br>±15·7 | 69·1<br>±12·2 | 637±85 |
| | Day 18 | 74·3<br>±5·8 | 105·2<br>±15·2 | 89·4<br>±16·1 | 75·6<br>±14·5 | 72·9<br>±12·6 | 688±82 |
| | Day 27 | 76·4<br>±7·2 | 102·6<br>±20·0 | 83·6<br>±19·7 | 75·1<br>±14·6 | 70·6<br>±12·4 | 670±103 |
| Mean serum insulin ±SD | Day 9 | 7·5<br>±3·7 | 55·4<br>±24·4 | 58·9<br>±23·7 | 47·7<br>±21·1 | 48·1<br>±25·1 | 380±129 |
| | Day 18 | 6·7<br>±2·0 | 59·8<br>±24·8 | 60·4<br>±28·6 | 53·5<br>±22·5 | 45·2<br>±23·1 | 395±144 |
| | Day 27 | 7·5<br>±2·7 | 61·7<br>±25·5 | 61·7<br>±25·8 | 48·9<br>±24·9 | 44·9<br>±32·7 | 397±158 |

differences between days of a cycle were established.

*Repeat studies*

These were performed three cycles later on seven subjects chosen at random from the twenty initially tested. The individual values are shown in Table III. There were no significant differences in blood sugar-time and insulin-time areas between the two cycles.

*Growth hormone* (GH)

The coefficient of inter-assay variation was 6·9 per cent. The mean fasting GH level on day 9 was 14·1 μu/ml. (S.E.±3·5); day 18, 11·7 μu/ml. (S.E.±2·6) and day 27, 15·2 μu/ml. (S.E.±3·5). Eleven subjects (55 per cent) demonstrated a rise in fasting GH levels at the time of impending menstruation but the difference in mean values was not statistically significant.

## DISCUSSION

Difficulty in controlling diabetes during menstruation was noted by Harrop and Mosenthal (1918), and Rosenbloom (1921). Greene (1958) reported a case of menstrual insulin resistance successfully treated with fludrocortisone and postulated that this suppressed an excessive growth hormone effect. Recently, Howel Walsh and O'Sullivan (1973), have drawn attention to the absence of variation in serum GH or plasma cortisol levels in insulin-requiring diabetics, although marked fluctuations in GH levels have been observed by others (Sabeh *et al.*, 1969; Hanssen, 1974). The rise in fasting serum GH level at the time of impending menstruation in eleven of the present series confirms the considerable individual variation in fasting GH levels, and it remains speculative whether a premenstrual rise in nocturnal or fasting levels can adversely affect glucose tolerance in diabetic women on fixed dose insulin regimes.

Early reports on menstrual cycle carbohydrate metabolism in normal women were conflicting. Okey and Robb (1925) noted that the fasting blood sugar was highest just before menstruation and lowest in the midcycle, whereas Billig and Spaulding (1947) postulated the possibility of premenstrual hyperinsulinism to account for their findings of a lower premenstrual blood sugar compared to the midcycle values. Spellacy *et al.* (1967) failed to observe any significant change in blood glucose and serum insulin levels

TABLE III

*Individual blood sugar and serum insulin levels, plus calculated blood sugar and insulin-time area values for 7 normal women who had repeat studies*

| Subject | Time (minutes) | | | | | Area | |
|---|---|---|---|---|---|---|---|
| | 0 | 30 | 60 | 90 | 120 | GTT | Insulin |
| | | | Day 9 | | | | |
| 7 | 93 | 117 | 88 | 79 | 79 | 740 | |
| | 6·6 | 46·5 | 42·5 | 47·7 | 49·8 | | 330 |
| 9 | 82 | 117 | 84 | 75 | 72 | 706 | |
| | 10·7 | 98·7 | 109·5 | 79·4 | 59·2 | | 645 |
| 12 | 67 | 120 | 125 | 75 | 84 | 791 | |
| | 6·8 | 88·1 | 83·1 | 73·0 | 45·0 | | 540 |
| 13 | 73 | 112 | 130 | 110 | 73 | 850 | |
| | 7·9 | 43·4 | 69·2 | 67·6 | 45·1 | | 413 |
| 14 | 76 | 129 | 85 | 76 | 66 | 722 | |
| | 11·2 | 113·2 | 114·2 | 94·0 | 90·1 | | 744 |
| 16 | 70 | 67 | 62 | 64 | 63 | 519 | |
| | 6·3 | 34·4 | 25·4 | 36·1 | 30·4 | | 228 |
| 19 | 84 | 108 | 80 | 74 | 76 | 684 | |
| | 5·9 | 43·2 | 29·5 | 23·4 | 24·8 | | 223 |
| | | | Day 18 | | | | |
| 7 | 77 | 120 | 95 | 90 | 87 | 774 | |
| | 14·8 | 69·3 | 55·4 | 70·9 | 70·4 | | 477 |
| 9 | 79 | 102 | 84 | 74 | 84 | 683 | |
| | 8·4 | 85·6 | 35·5 | 35·0 | 36·0 | | 357 |
| 12 | 70 | 111 | 90 | 68 | 65 | 673 | |
| | 6·7 | 64·1 | 73·2 | 65·1 | 55·9 | | 467 |
| 13 | 85 | 110 | 93 | 72 | 64 | 699 | |
| | 5·4 | 64·9 | 66·9 | 28·5 | 23·4 | | 349 |
| 14 | 81 | 145 | 122 | 94 | 85 | 888 | |
| | 10·8 | 108·4 | 110·3 | 91·2 | 85·2 | | 716 |
| 16 | 71 | 65 | 72 | 70 | 64 | 549 | |
| | 10·7 | 24·9 | 46·6 | 43·4 | 22·8 | | 262 |
| 19 | 76 | 111 | 99 | 70 | 67 | 703 | |
| | 6·1 | 41·7 | 55·7 | 55·9 | 12·6 | | 325 |

TABLE III—*continued.*

| Subject | Day 27 | | | | | | |
|---|---|---|---|---|---|---|---|
| | Time (minutes) | | | | | Area | |
| | 0 | 30 | 60 | 90 | 120 | GTT | Insulin |
| 7 | 84 | 115 | 90 | 64 | 72 | 694 | |
| | 10·9 | 26·5 | 25·2 | 42·8 | 46·3 | | 246 |
| 9 | 75 | 101 | 79 | 72 | 68 | 647 | |
| | 8·9 | 142·6 | 149·2 | 154·1 | 101·4 | | 1002 |
| 12 | 71 | 105 | 79 | 64 | 51 | 619 | |
| | 9·1 | 92·3 | 82·1 | 60·7 | 48·1 | | 527 |
| 13 | 73 | 94 | 65 | 68 | 52 | 579 | |
| | 5·1 | 53·8 | 39·9 | 36·0 | 29·7 | | 294 |
| 14 | 77 | 128 | 106 | 87 | 79 | 798 | |
| | 7·3 | 63·1 | 63·4 | 55·8 | 54·1 | | 426 |
| 16 | 81 | 95 | 79 | 82 | 79 | 672 | |
| | 9·5 | 47·1 | 35·6 | 29·8 | 35·4 | | 270 |
| 19 | 85 | 109 | 95 | 79 | 75 | 726 | |
| | 9·1 | 28·1 | 59·2 | 32·6 | 43·6 | | 293 |

after *intravenous* glucose in nineteen subjects on days 5 and 25 of the cycle. Goldman and Eckerling (1970) similarly did not observe any change in blood sugar levels in fifteen women on days 14 and 24 of the menstrual cycle following *intravenous* glucose loading.

Jarrett and Graver (1968) carried out repeated *oral* glucose tolerance tests over one or two cycles in ten women aged 19 to 39 years (three were multiparae), and noted that a pronounced variation in glucose handling occurred in seven subjects. The effect of age on glucose tolerance was demonstrated by Boyns *et al.* (1969), and Howel Walsh and O'Sullivan (1973) recently confirmed that variations in glucose handling in insulin-requiring female diabetics was a feature related to subjects over the age of 35 years and not associated with any particular phase of the menstrual cycle.

It can be concluded from the present study that there is both wide inter- and intra-individual variation in glucose tolerance and insulin response in repeated oral glucose tolerance tests, despite rigid control of the test procedure and the elimination of variable factors such as age and parity. It can also be concluded that in a group of normal nulliparous women of similar age, these variations do not exceed the accepted criteria of normality, and furthermore, there is no recognizable pattern which would suggest a common intra-menstrual cyclic variation secondary to known changes in endogenous hormone levels.

It is speculated that specifically designed statistical models may be employed to detect alterations in both glucose tolerance and insulin response in subjects whose glucose homeostatic control may be suspect, for example, during pregnancy or oral contraceptive therapy and in potential diabetics. Billewicz *et al.* (1973) have recently proposed the use of the "H" index which describes the shape of the oral glucose tolerance test response curve, to detect small changes in glucose handling despite the presence of normal blood sugar levels.

It has been suggested (Jarrett and Graver, 1968) that tolerance is greatest in the early phase of the cycle during the time when endogenous oestrogen levels are low, and least at about the time of ovulation. Lorraine and Bell (1963) demonstrated peaks of urinary oestrogen excretion towards the midcycle and also during the luteal phase. Studies on the effects of exogenous oestrogen and progesterone on carbohydrate metabolism have been made by Goldman *et al.* (1968) and Goldman and Ovadia (1969), who concluded that oestrogen rather than progestogen reduced glucose tolerance; but Gershberg *et al.* (1967) have reported an improvement in carbohydrate metabolism in some patients with maturity onset diabetes after treatment with oestrogens.

There is a recent trend to low dose combined oral contraceptive compounds with an oestrogen content of 30 μg. (Bye and Elstein, 1973). Investigations into the effects of these steroids on carbohydrate metabolism reinforce the need for adequate control data on any cyclic changes in glucose and insulin levels in normal women. Prospective studies on the metabolic effects of oral contraceptives should be designed to reduce the influence of such variables as age, body weight and parity.

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# PNEUMOTHORAX AND SURGICAL EMPHYSEMA IN A NEWBORN BABY CAUSED BY AMNIOCENTESIS

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## Summary

A report is presented of a newborn baby who developed a pneumothorax and extensive surgical emphysema after being delivered by Caesarean section for fetal distress soon after the mother had an amniocentesis.

TRANSABDOMINAL amniocentesis is being applied to an increasing number of antenatal problems. Although the risk is small there are few reports of severe complications to the fetus (Creasman *et al.*, 1968). We describe such a case.

## CASE REPORT

A twenty-three-year-old woman who had had one previous pregnancy was admitted at 40 weeks gestation by dates but only 36 weeks by size for amniocentesis as an aid in the assessment of gestational age (Droegenmueller *et al.*, 1969). The amniotic fluid was found to be heavily stained with meconium. Labour was induced by artificial rupture of the membranes but because of persistent fetal tachycardia of 180 beats per minute a Caesarean section was performed. A term male infant weighing 2·11 kg. was delivered; it had an Apgar score of 8, and did not require endotracheal intubation. There were a number of puncture marks in the skin over the right side of the chest posteriorly, but no other abnormalities were detected. When one hour old, the child developed respiratory difficulty with grunting, subcostal recession and tachypnoea, but no cyanosis. A large area of surgical emphysema (Fig. 1) had developed on the same side of the chest wall as the puncture marks, movement was minimal and air entry reduced. A chest X-ray showed the presence of a large pheumothorax and confirmed the presence of a considerable surgical emphysema (Fig. 2). An Argyle catheter FG 12 was inserted into the second right intercostal space anteriorly and connected to a Hermlich valve (Ramachandra and Russell, 1972). A repeat chest X-ray an hour later showed complete resolution of the pneumothorax but the surgical emphysema persisted, the latter gradually resolving in the course of the next three days. The infant's condition remained satisfactory during the rest of his stay in the Special Care Baby Unit and two months later he was thriving and had no clinical abnormalities.

## COMMENT

Surgical emphysema was not present in the previously reported case of pneumothorax complicating amniocentesis (Creasman *et al.*, 1968) and the authors were, therefore, only "highly suspicious that the amniocentesis was the aetiological factor . . . ". The case reported in this paper demonstrates that the pneumothorax was due to the trauma sustained at amniocentesis, for surgical emphysema was limited to the area overlying the right side of the chest wall. The amniocentesis needle must have punctured the skin, the chest wall, both layers of the pleura and the lung.

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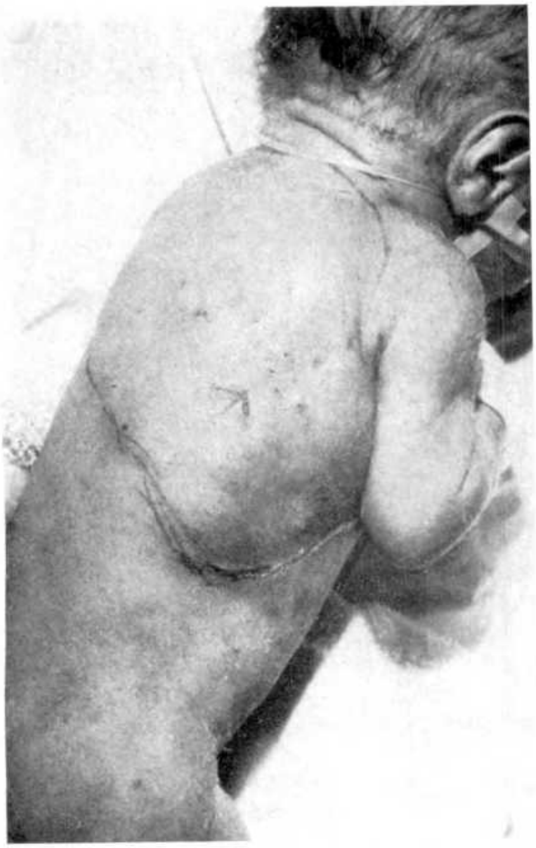

FIG 1
Surgical emphysema (outlined by ball pen lines) and amniocentesis puncture marks (arrowed) on skin.

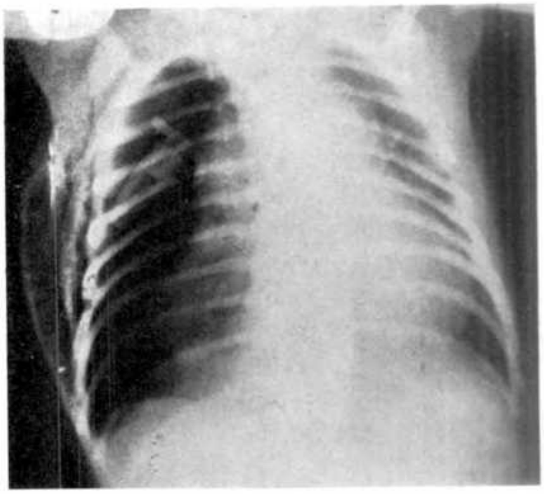

FIG. 2
Anteroposterior chest X-ray showing right pneumothorax and surgical emphysema.

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I thank Mr. A. Bushby for allowing me access to the mother's notes and permission to publish this case.

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# MATERNAL CARDIOMYOPATHY OF PREGNANCY CAUSING STILLBIRTH

BY

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## Summary

Cardiomyopathy of pregnancy developed in a healthy primigravida at 34 weeks gestation and was followed two weeks later by the spontaneous delivery of a fresh stillbirth. An immunological basis underlying both the mother's condition and the fetal death was suggested by the presence of myocardial antibodies in maternal and cord blood. Reference is made to two other cases in which a similar pathological process may have occurred.

Although there have been many reported cases of cardiomyopathy of pregnancy and the puerperium since the disease was first described in 1849 (Meadows, 1960), and in spite of the recognition that over 80 per cent of premenopausal women developing cardiomyopathy do so in relation to pregnancy (Seftel and Susser, 1961; Stuart, 1968; Burch *et al.*, 1971), there is still doubt about its precise nature but an immunological basis seems at least a possibility. On the evidence presented in our case report it appeared possible that immunological factors were operative in both the maternal cardiomyopathy and in the fresh stillbirth that occurred two weeks later.

## Case Report

The patient was a 27-year-old married primigravida with no personal or family history of heart disease or hypertension. She was prone to minor nose bleeds. From the 16th week of pregnancy onwards she had recurrent severe epistaxes requiring hospital admission on three occasions. However, the prothrombin and kaolin/cephalin times were normal; the platelet count was 253,000 per cu. mm. with normal morphology; the differential white cell count was normal and the erythrocyte sedimentation rate was 92 mm. in the first hour. The blood urea and electrolytes were normal. Total serum protein levels were normal but plasma electrophoresis demonstrated raised levels of gamma globulin and reduced albumin. The fibrinogen degradation products concentration in peripheral blood was greater than 10 $\mu$g./ml. and therefore outside the normal range for early pregnancy. This abnormality persisted until after delivery. By 27 weeks her haemoglobin had fallen to 7·9 g./100 ml. despite haematinic therapy and was transfused with 3 litres of blood.

At 29 weeks her blood pressure was recorded as 170/90 mm. Hg. She was therefore admitted to hospital where she remained until after delivery. However, the blood pressure quickly settled to 140/90 mm. Hg or less and there was no associated albuminuria. Serial measurements of 24 hour urinary oestriol levels and of the fetal biparietal diameter by ultrasound were normal.

At 34 weeks gestation acute heart failure developed with severe dyspnoea and oedema. There was a loud first heart sound and two distinct systolic murmurs, one of which was best heard at the apex and radiated to the axilla and the other of which was loudest down the right sternal edge and radiated to the clavicle. There was no pyrexia, chest pain, cough, or sign of deep vein thrombosis. An X-ray showed marked cardiomegaly with an elevated right hemidiaphragm and dilated upper pulmonary veins. There was no recent lung lesion, nor other cause for the enlarged heart. An electrocardiograph showed sinus tachycardia but was otherwise normal. The haemoglobin was 11·2 g./100 ml.; the white cell count was 6,900 cu. mm.; the blood film and platelets were normal; the erythrocyte sedimentation rate was 55 mm. in the first hour; and the blood urea and electrolytes were all normal apart from a slightly low serum potassium of 3·6 mEq./l. The serum albumin was 2·4 g./100 ml. and total serum proteins were normal. As in early pregnancy plasma electrophoresis demonstrated normal levels of alpha-1, alpha-2 and beta-globulins, but raised gammaglobulin, and reduced albumin. The 24-hour excretion of catecholamines was not elevated. Thyroid function was normal as assessed by the protein bound iodine, and tri-iodothyronine and tetraiodothyronine uptakes. Virus antibody titres against *Myxovirus influenzae A* and *B*, *adenovirus*, *psittacosis virus*, *coxiella burnetti*, *Mycoplasma pneumoniae* and Coxsackie viruses $B_1$, $B_2$, $B_3$, $B_4$, $B_5$, and $B_6$ were all low and they did not rise in the course of the following two weeks. Antimyocardial sarcolemma antibodies were detected in the peripheral blood in a titre of 1 in 45.

Cardiomyopathy of pregnancy was diagnosed and treatment initiated with frusemide 40 mg. daily and potassium supplements. Within 24 hours 5 litres of urine were excreted and by the fifth day the cardiac failure had cleared.

Two weeks later, at 36 weeks gestation, premature labour commenced. After two hours of regular contractions a vaginal examination revealed the cervix was 7 cm. dilated. An amniotomy was performed releasing thickly meconium stained liquor amnii. At the same time the fetal heart rate became irregular with severe bradycardia between contractions. Despite prompt delivery with the ventouse extractor, the baby, a boy weighing 2·32 kg., was stillborn.

No physical disturbance followed the delivery. The patient was mobilized early and discharged at six days *post partum*. The serum potassium was 3·6 mEq./l. and serum albumin 2·2 g./100 ml. Plasma electrophoresis showed normal gammaglobulin levels. Fibrinogen degradation products were still present in the peripheral blood.

Six weeks later the patient was extremely well. The heart was no longer enlarged on screening and the electrocardiograph was normal.

### *Morbid anatomy*

Autopsy of the baby showed no macroscopic abnormalities. Sections of the heart showed unusually eosinophillic and homogeneous myocardial cytoplasm; cytoplasmic outlines were irregular and blurred with complete loss of striations. Between the myocardial fibres there were wide spaces filled with faintly eosinophillic fluid. The myocardial nuclei, however, were preserved, and an inflammatory response was evident. The appearances were consistent with the influence of an antibody.

### *Immunological studies*

The results of immunoglobulin estimations made *at delivery* are shown in Table I. The cord serum was positive in a titre of 1 in 15 for myocardial sarcolemma antibodies identical to those detected in the maternal blood.

TABLE I

*Immunoglobulin levels at delivery and their normal range*

| | Type of immunoglobulin | Value at delivery (mg./100 ml.) | Normal range (mg./100 ml.) |
|---|---|---|---|
| Cord blood | IgG | 1150 | 1077–1839 |
| | IgM | 13 | 8·2–23·0 |
| Maternal blood | IG | 1900 | 300–1600 |
| | IgA | 640 | 140–420 |
| | IgM | 125 | 50–200 |

Sections of the heart and kidney were studied by direct immunofluorescence with fluorescein labelled antisera to human immunoglobulins, and indirectly with antisera to human complement. IgG was irregularly present throughout the heart, linearly distributed alongside scattered muscle fibres in the sarcolemma and adjacent connective tissue fibrils, and in the sarcoplasm of occasional myofibres. No IgA, IgM or complement was detected. No additional fixation of IgG was noted when sections were reacted with maternal serum prior to reaction with fluorescein labelled anti-IgG antisera, although the maternal serum was shown to contain immunoglobulins capable of fixing to the sarcolemma and subsarcolemmal sarcoplasm of rat heart. No renal immunohistological abnormality was noted.

The patient and her husband were O Rhesus positive. The HL-A types of mother and husband were reported HL-A3, 12, W10 and HL-A1, 9, 8 respectively when tested against 20 antisera.

## Discussion

The incidence of cardiomyopathy of pregnancy and the puerperium has been quoted as between 1 in 1300 and 1 in 4000 confinements (Meadows, 1957; Woolford, 1952; Seftel and Susser, 1961), accounting for up to 5 per cent of cardiac deaths in pregnancy (Gouley *et al.*, 1937). However, many authors do not believe this is a specific cardiomyopathy, but that the strain of pregnancy precipitates failure in a previously damaged heart (Bashour and Winchell, 1954; Benchimol *et al.*, 1959; Gilchrist, 1963; Robinson *et al.*, 1970; Hudson, 1972). Superadded hypertension may be an important aetiological factor, and is said to occur in up to 30 per cent of cases (Pierce *et al.*, 1963).

It is interesting that this patient had a high erythrocyte sedimentation rate, circulating fibrinogen degradation products and raised gamma globulins four months before her acute illness. She subsequently developed mild pre-eclampsia and this may have precipitated the cardiac failure. The frequent severe epistaxes were unexplained but abnormal bleeding has been described with cardiomyopathy of pregnancy (Hull and Hafkesbring, 1937; Meadows, 1957).

The studies on the baby's heart indicate a primary pathology rather than terminal anoxic or postmortem changes. It is possible that damage to both the maternal and fetal myocardium was caused by a common agent, such as a virus. Cases are reported in which mothers have had a viral infection a few days before labour, and the baby has subsequently died of myocarditis. In some cases virus has been isolated from the baby's myocardium (Javett *et al.*, 1956; Kibrick and Benirschke, 1956; Van Creveld and De Jager, 1956; Delaney and Fukunaga, 1958; Woodward *et al.*, 1960; Doshi and Lodge, 1973). However, in our case the maternal serum antibody titres estimated during the third trimester gave no evidence of recent viral infection, and the cord blood immunoglobulin levels did not suggest intrauterine viraemia.

Sanders (1963) drew attention to the striking similarity between chronic infectious myocarditis and a large group of unexplained myocardial diseases, and he discussed the possible role of autoimmunity in their development. Other authors have suggested that the production of myocardial antibodies and the histology of affected hearts indicate that cardiomyopathy of pregnancy and the puerperium may be a primary autoimmune disease (Becker and Taube, 1962; Hudson, 1972). However, heart antibodies are not invariably present in this condition (Hughes *et al.*, 1970; Robinson *et al.*, 1970), and they are found in patients with rheumatic fever, and following myocardial infarctions or cardiac surgery (Kaplan and Frangley, 1969). Indeed antibody formation has been described as "the common denominator in myocardial disease" (Editorial, 1962). Therefore, whether the high titre of antimyocardial sarcolemma antibodies in this patient was the primary cause of her illness or secondary to underlying disease is uncertain.

Heart antibodies in cord blood have not to our knowledge been recorded. Nevertheless, if maternal blood contains such IgG antibodies they will cross the placental barrier and the fetus is likely to be affected. This mechanism could explain the intrauterine death and the subsequent histological and immunological findings.

Most case reports of cardiomyopathy of pregnancy fail to mention whether or not the baby survived. However, one of 15 patients described by Brown *et al.* (1967) was almost identical to ours. Having responded to treatment for her unexplained cardiac failure, she went into spontaneous labour and had a fresh stillbirth after the fetal heart had stopped without warning during the second stage. A similar immunological explanation could account for the autopsy finding of cardiomyopathy on a baby who died a few minutes after delivery by postmortem Caesarean section, the mother having died of cardiac failure attributed to cardiomyopathy developing for the first time during the third trimester of her second pregnancy. This mother had no history of overt heart disease but two years earlier her first baby had died in infancy of cardiomyopathy (Redman, 1974). It is possible that she had an underlying autoimmune cardiomyopathy from the time of her first pregnancy and that both infant deaths were due to passively acquired antibodies.

Our findings suggest that immunological factors may play a role in the pathogenesis of cardiomyopathy of pregnancy and that there may be a transplacental influence on the fetus. This would represent a situation analogous to neonatal myasthenia gravis, thyrotoxicosis, and thrombocytopenic purpura in which auto-antibodies transmitted across the placenta cause disease in the baby. The effect, if not fatal, may be transient, clearing in the weeks following birth (Scott, 1966).

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# BOOK REVIEW

**Human Reproduction and Society.** By D. LLEWELLYN-JONES. Pp. 547. Faber and Faber, London, 1974. Price: £5.25.

MANY of the world's troubles stem from its increasing overpopulation. The book deals with the social and demographic problems of human reproduction. In the first section, on human dynamics, the many factors which cause the growth and composition of population are considered. Birth rate is the basis of overpopulation and is affected by such diverse factors as age at first intercourse, attitudes to marriage and divorce, migration, social class and education. Apart from these tangible influences. there seems also to be ill-understood vagary in human reproductive behaviour which has often caused errors in population predictions in the past and may well do so in the future. Even allowing for errors in prediction, it is clear that the increased use of our free production resources cannot keep pace with the exponential growth in the number of people. Survival can only lie in population control by family planning in its widest sense. Zero population growth is the first aim but, even when achieved, the population momentum is such that a further 35 to 60 years is needed before a stationary population results. On the basis of all nations having achieved the zero population growth by 1995, the world's population will finally stabilize at over 7400 million people in the year 2060. Of these, 6000 million will live in the currently developing nations and 1400 million in the affluent nations. The second section of the book deals with the technology of birth control and covers that more familiar ground of contraception, abortion and sterilization. The integration of all methods of fertility control into family welfare is considered in a separate chapter which also deals with the goals of birth control campaigns and their evaluation. At a time when much money is being invested in family planning, evaluation results are especially important. It is salutary to consider the success of China in evolving a cost-effective programme to control her large population. It is noteworthy that family planning there contains no element of compulsion but puts strong emphasis on the initiative and self reliance of the people themselves. In the final section of the book the author deals with the social factors in reproductive efficiency. In considering the causes of perinatal and maternal mortality it is important for the obstetrician to appreciate that social factors have a much greater influence than good obstetric care; this is not to detract from the value of such care but to underline the fact that in the end the quality of the reproductive "product" depends on the quality of the parents and the environment in which they live. Obstetricians should show greater concern to see that every woman is educated to avoid becoming a teenage mother (especially if unmarried), an elderly primigravida or a grand multipara, since these categories are characterized by poor reproductive efficiency and high mortality rates. In gathering together a wealth of information contained in this book, and presenting it in an extremely interesting and readable way, Professor Llewellyn-Jones has made a valuable contribution to obstetric and gynaecological literature which will be of great interest not only to the practising clinician but to all who are concerned with the social problems of human reproduction. *M. Brudenell*

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These should be avoided as far as possible. "Per cent" should be used instead of the symbol "%" and such forms as T.B., R.B.C., B.P., P.E.T., A.P.H. eschewed. Dates should be given as 10th May, 1974 and *not* 10.V.74. The official rather than the proprietary names of drugs must be used.

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1 **World Med., 8,** (19), 73, 1973
2 **Gen. Practit.,** p. 19, 13 July, 1973
3 **Practitioner, 212,** 115, 1974
4 **Br. Med. J.,** iv, 146, 1971
5 **Ibid.,** i, 207, 1971

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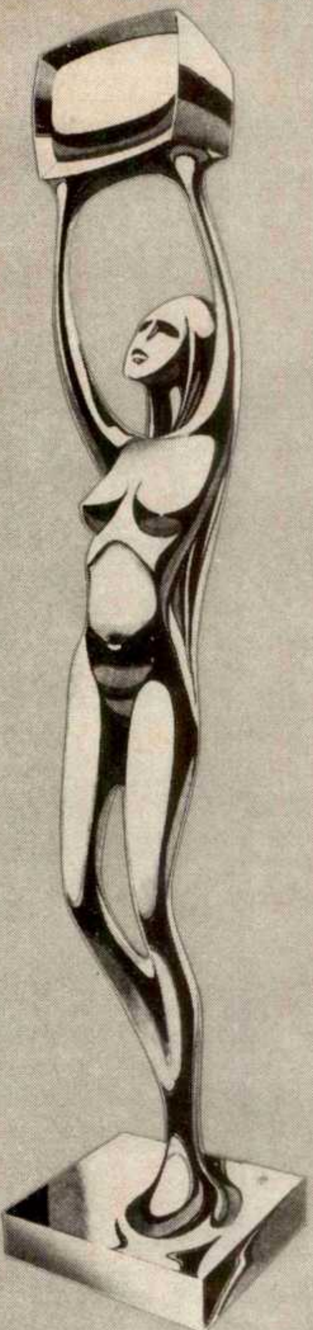

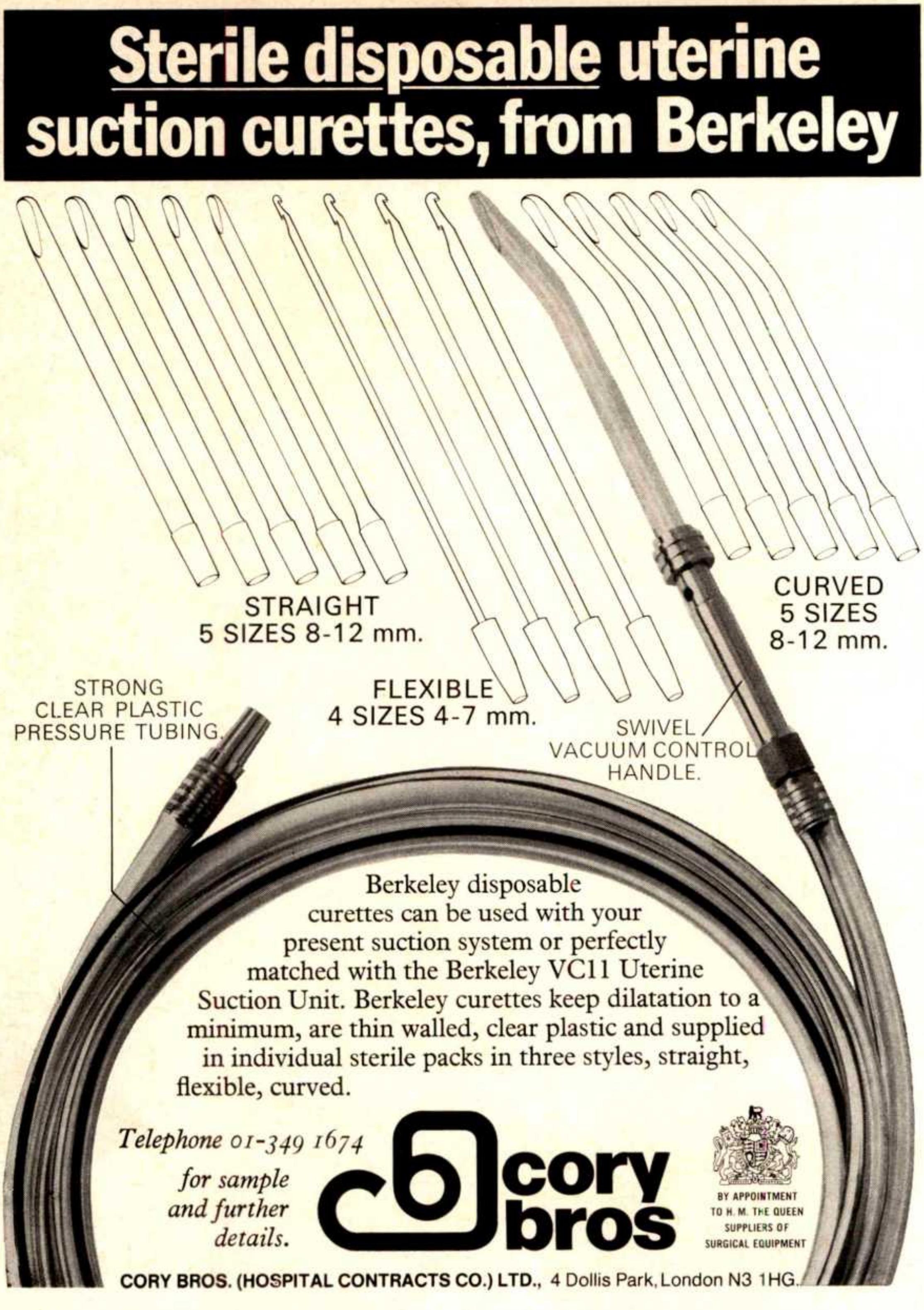

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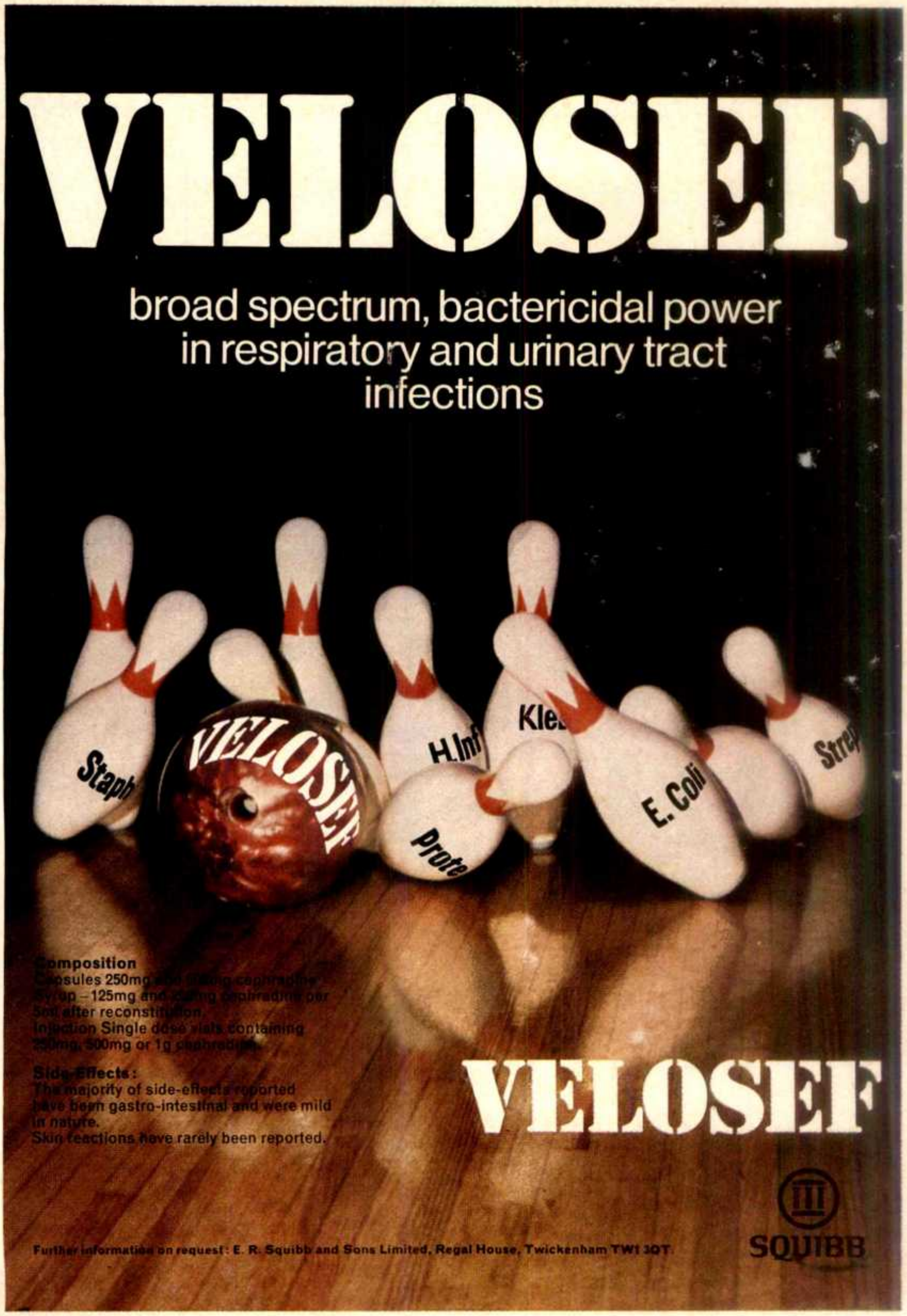
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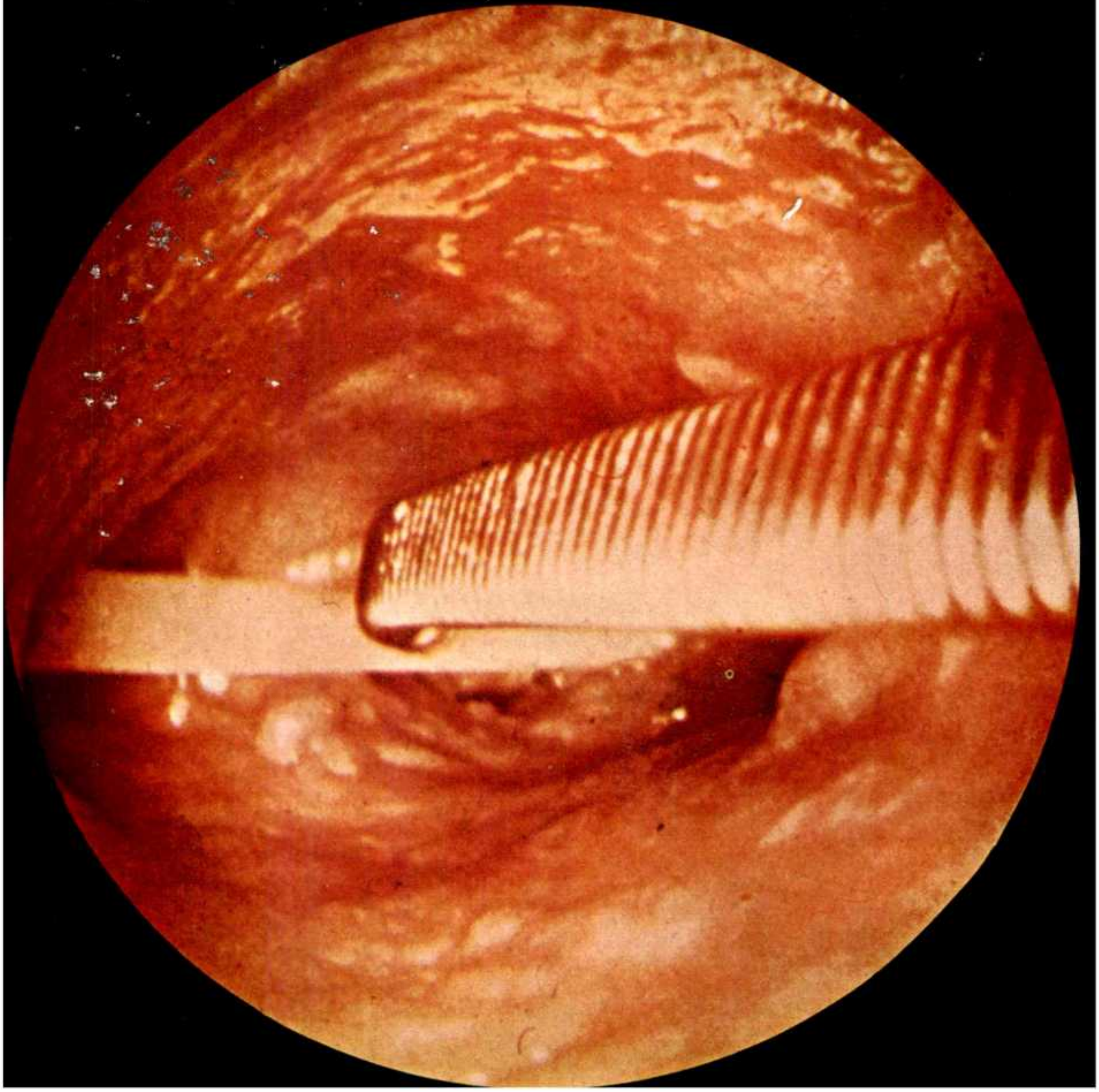

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| Progestogen-only pill[3] | 2·4 |
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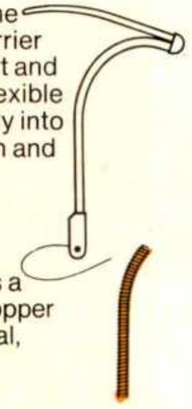

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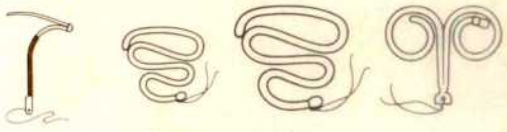

| **Gravigard**[2] | Lippes Loop C[4] | Lippes Loop D[4] | Saf-T-Coil[4] |
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[1] Human Reproduction and Society, Faber, 1974
[2] Brit. Med. J., 1974, **3,** 447–450
[3] Data on files of Searle Laboratories
[4] IPPF booklet "Intrauterine Contraception"
[5] Am. J. Obstet. Gynecol. 1973, **117,** 602

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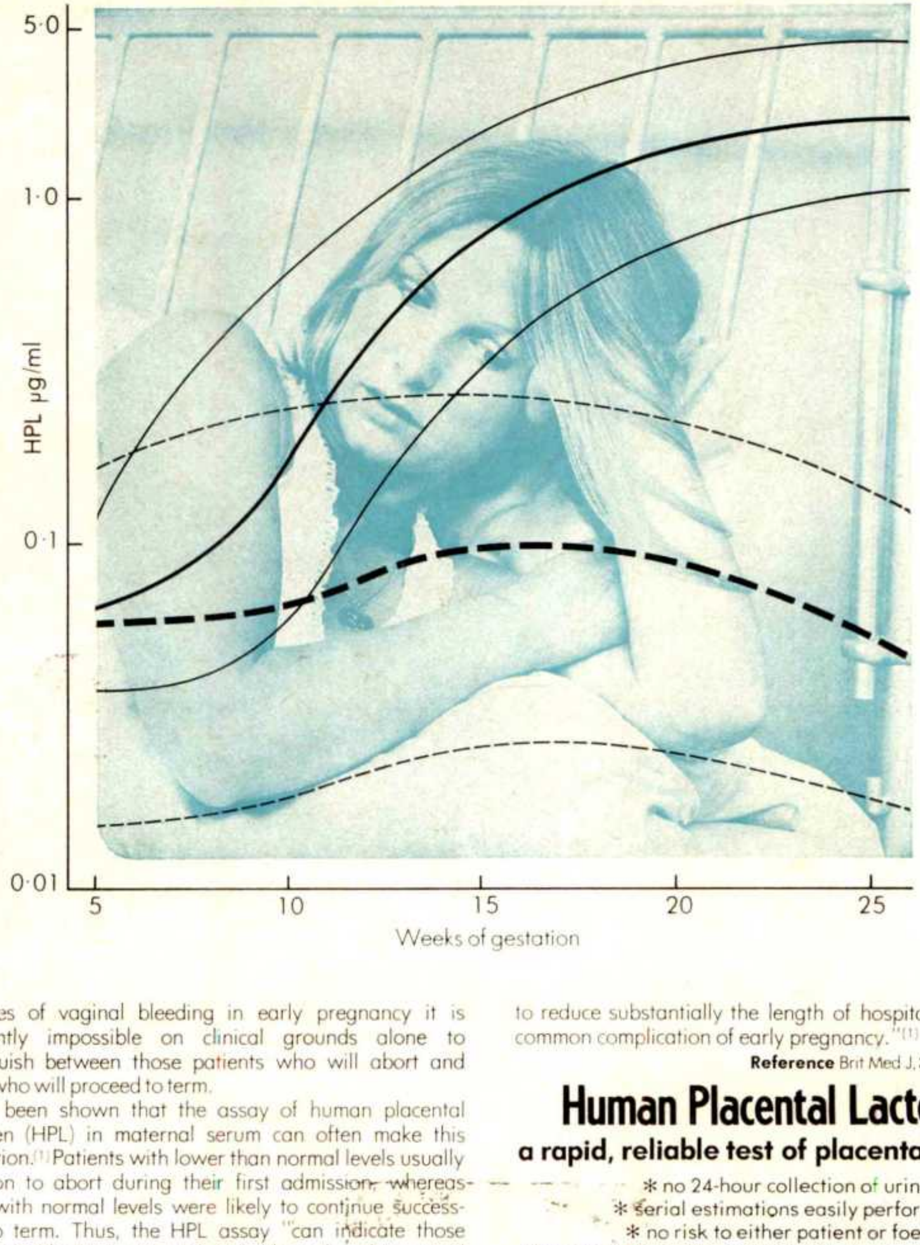

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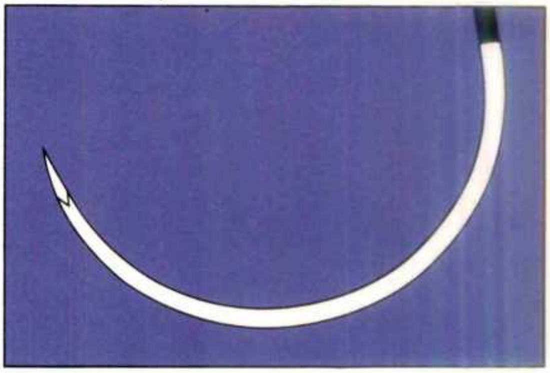

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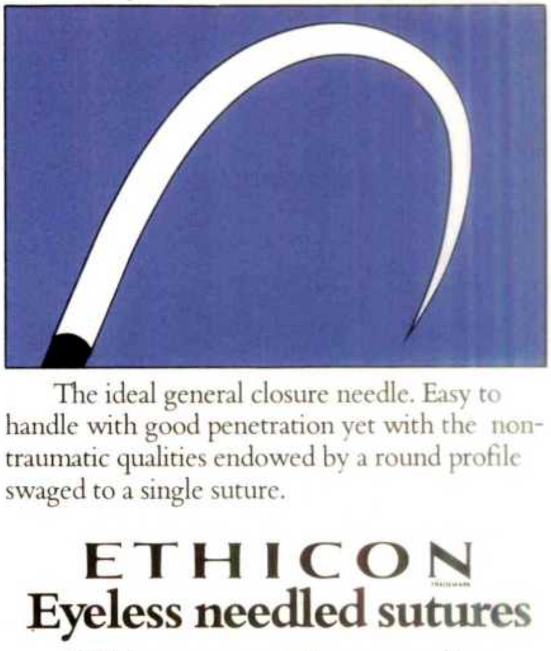

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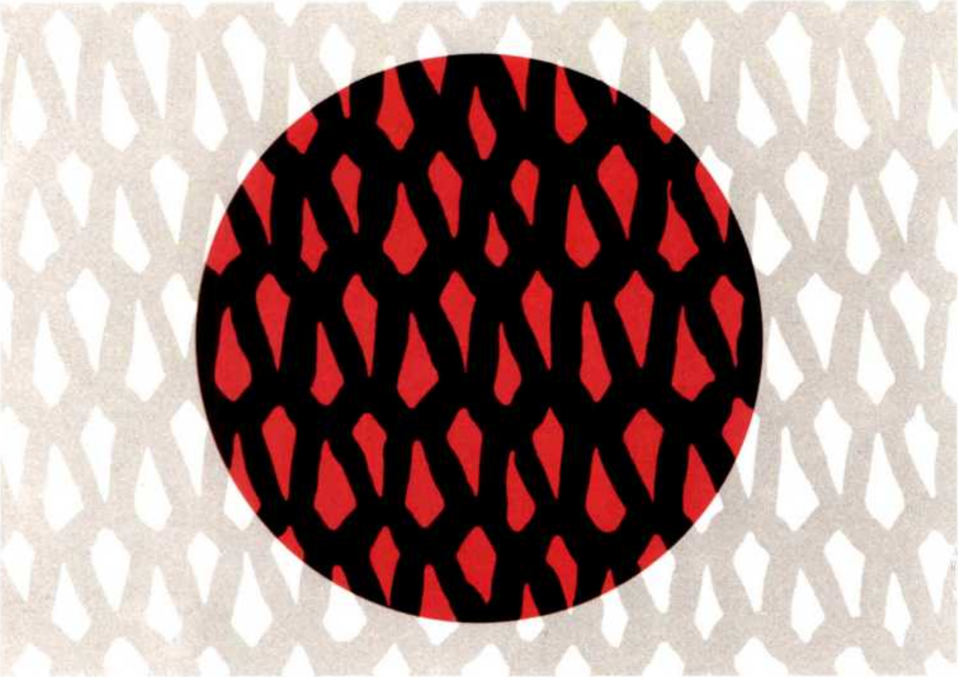

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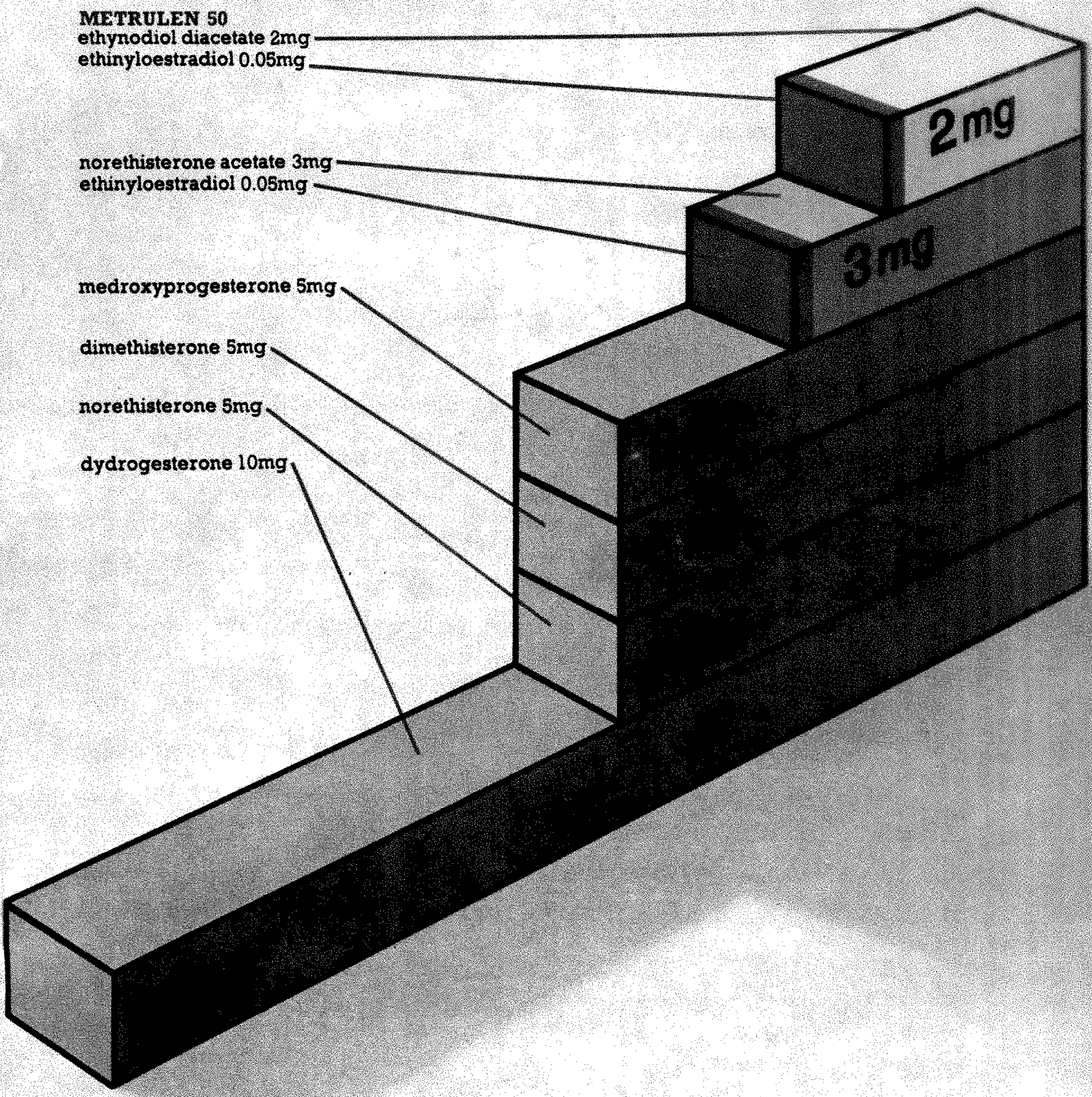

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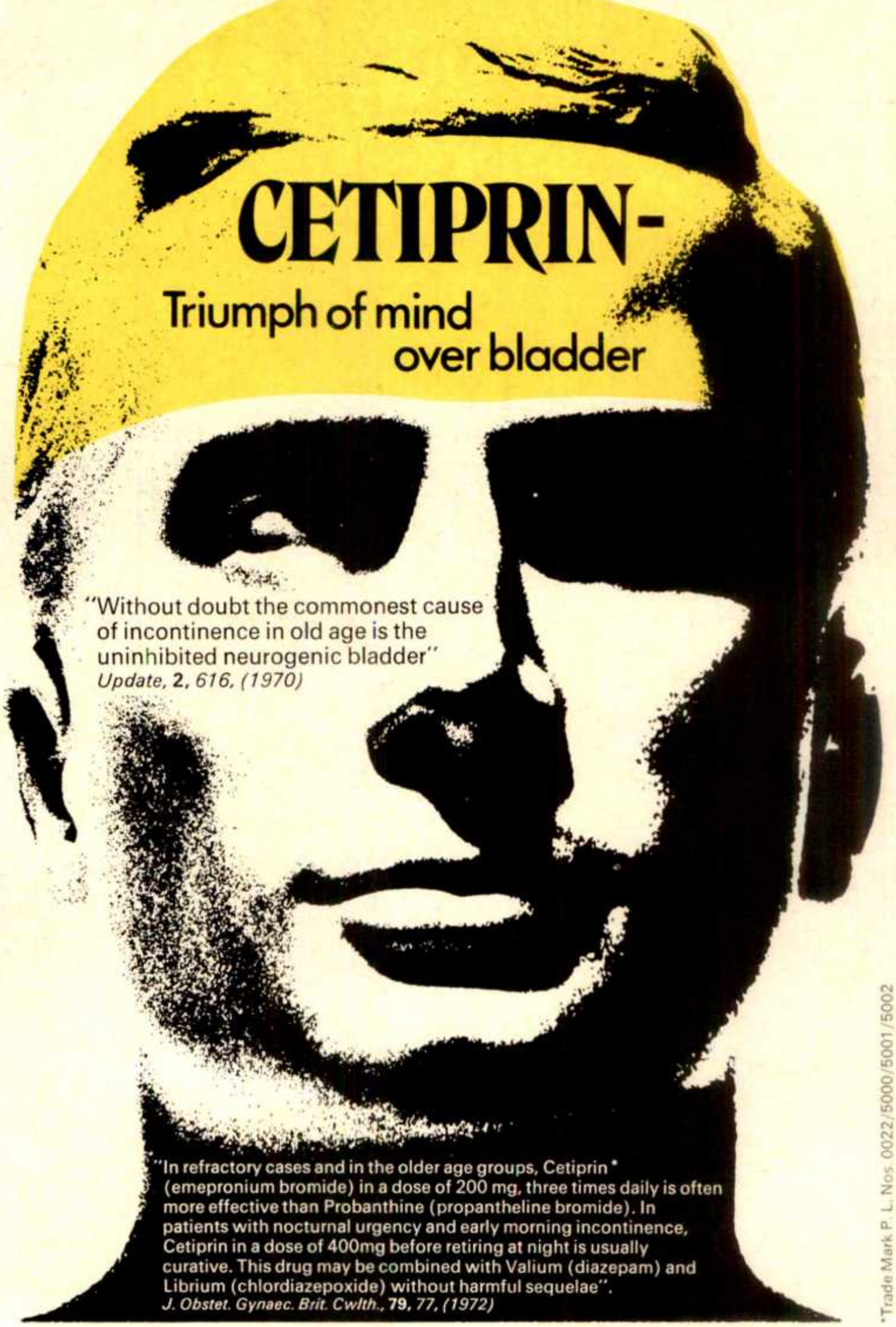

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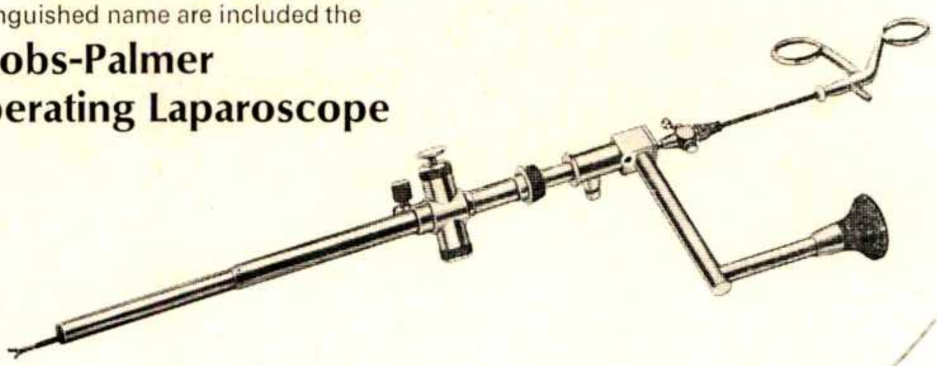

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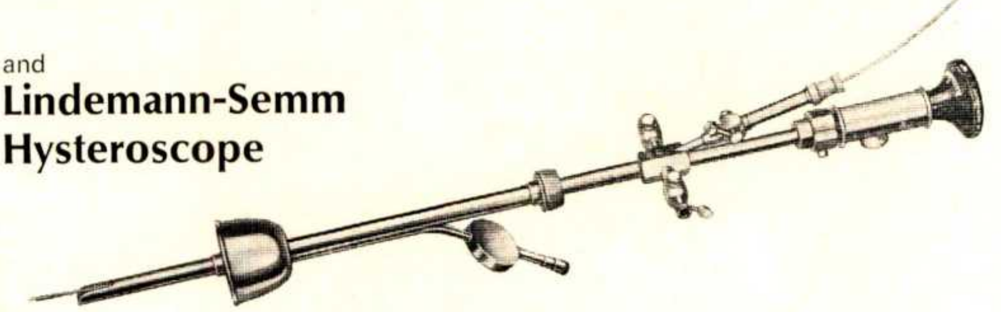

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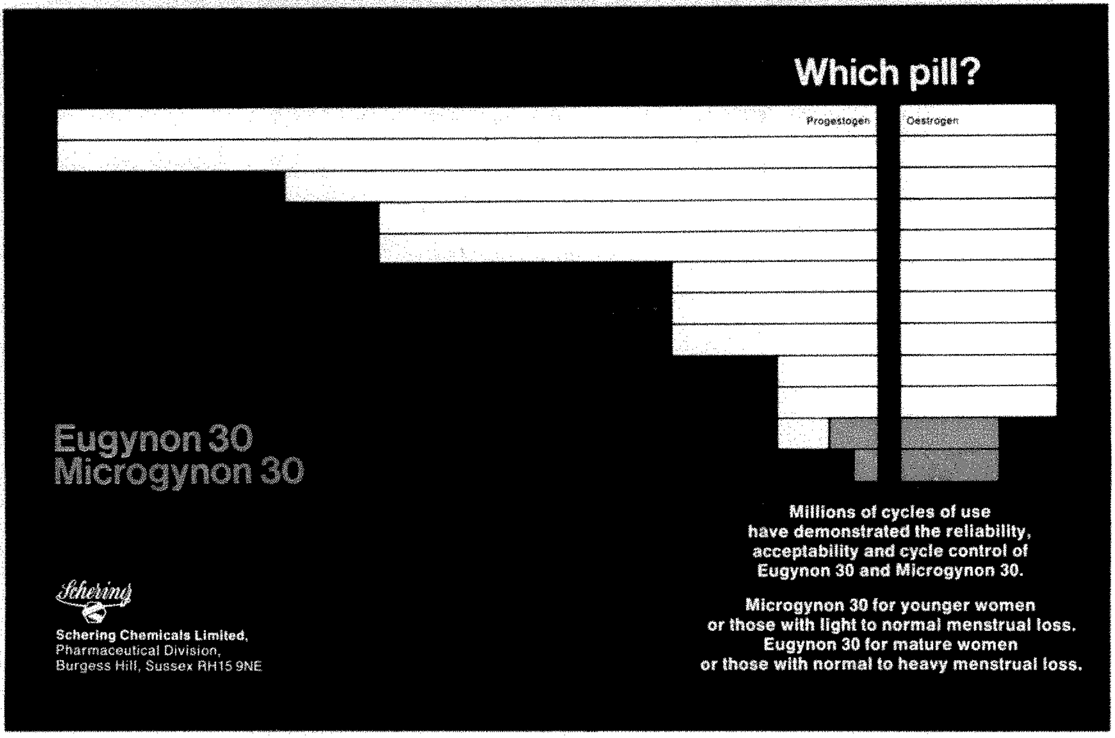

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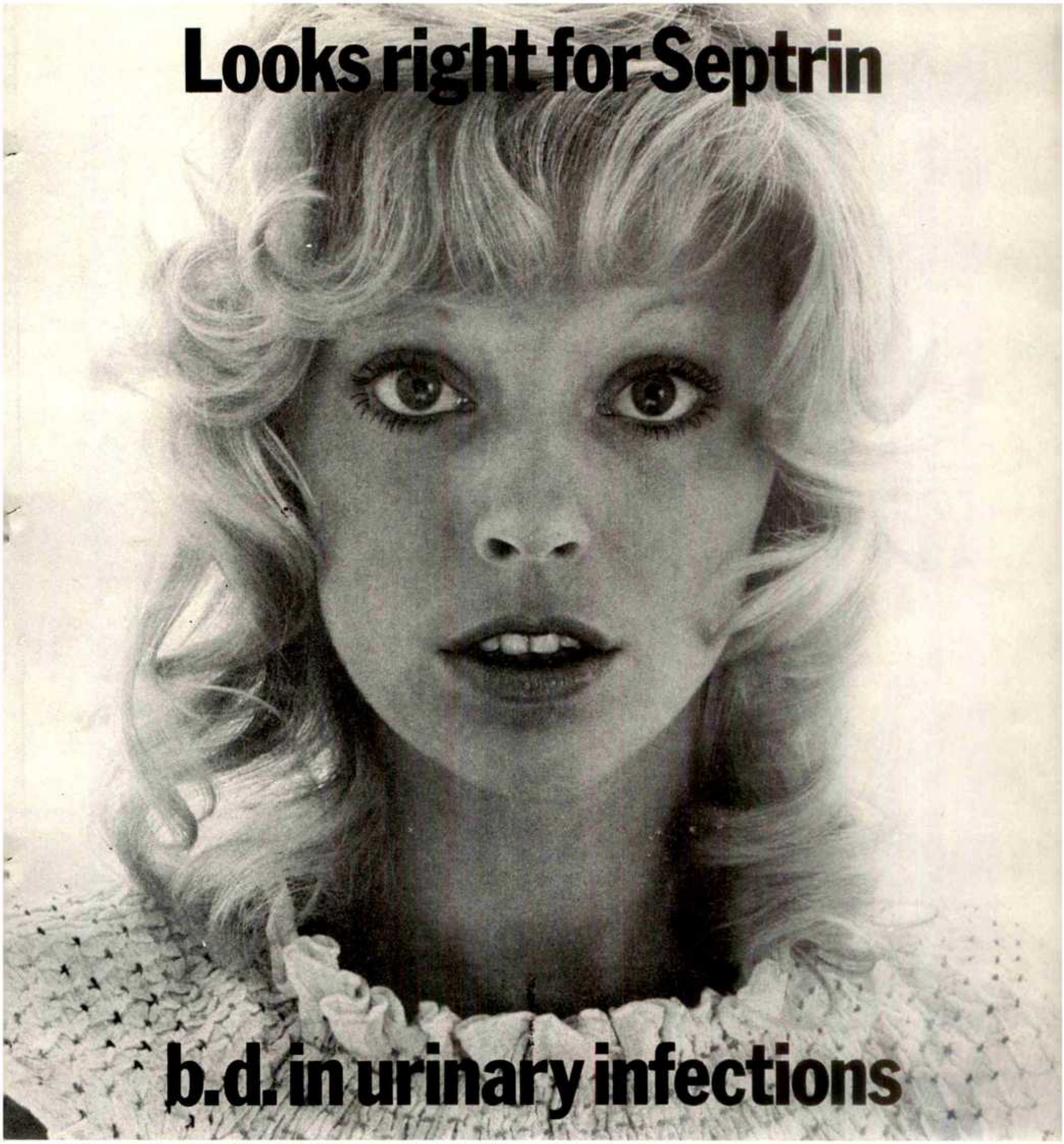

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1 *Postgrad med J*, (1971) **47**, Supplement (September), 7.
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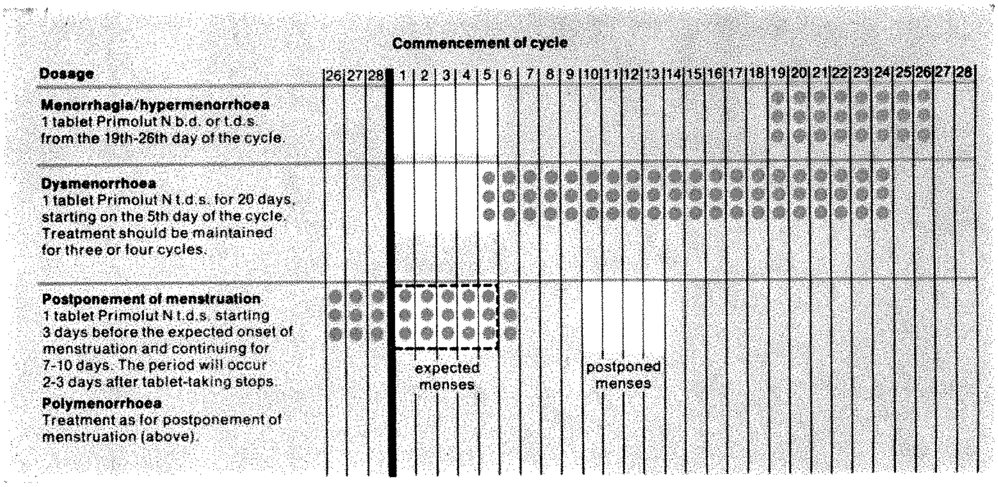

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British Journal of Obstetrics and Gynaecology

VOL. 82 No. 3 NEW SERIES MARCH 1975

# RELEASE OF LYSOSOMAL ACID PHOSPHATASE INTO THE CYTOPLASM OF DECIDUAL CELLS BEFORE THE ONSET OF LABOUR IN HUMANS

BY

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## Summary

In order to elucidate the mechanism triggering the onset of spontaneous labour, decidual cells obtained at elective Caesarean section at term were studied histologically and cytochemically. Although some of the cells were well preserved, many of them showed degenerative changes and signs of release of the lysosomal marker enzyme acid phosphatase into the cytoplasm indicating lysosomal labilization. Since human decidua contains lysosomal phospholipase $A_2$, an enzyme which specifically removes prostaglandin precursor acids from tissue phospholipids and thereby provides substrate for prostaglandin synthesis, the lysosomal labilization might be of significance in the initiation of labour. It is suggested that this labilization is induced by altered levels of sex steroids.

PROSTAGLANDINS are potent myometrial stimulants and can induce labour at term (Karim *et al.*, 1970), and abortion in the second trimester of pregnancy (Wiqvist *et al.*, 1973). During labour, high concentrations of prostaglandins have been found in decidual tissue (Karim and Devlin, 1967), and it has been assumed that prostaglandins play a physiological role in parturition (Karim, 1968). The mechanisms that initiate the synthesis of prostaglandins are not fully understood but the action of phospholipase $A_2$ released from lysosomes within decidual cells has been suggested as a trigger of prostaglandin synthesis in late pregnancy (Gustavii, 1972 and 1974). This enzyme is thought to cleave prostaglandin precursor acids from membrane phospholipids. A search of the literature failed to reveal any investigation of lysosomes in decidual cells at term before the onset of spontaneous labour. This paper reports a morphological and enzyme histochemical study of such cells.

## METHODS

Decidual tissue was obtained from 12 women at elective Caesarean section at term. Decidua obtained from 10 women at midpregnancy hysterotomy served as control material. After delivery, but before removal of the placenta and the fetal membranes, a piece of tissue, consisting of fetal membranes, decidua, and a few millimetres of myometrium, was excised. The tissue samples were divided into two parts; one part was used for morphological studies; the other, for cytochemical demonstration of the lysosomal marker enzyme acid phosphatase (Enzyme Commission 3.1.3.2.). The tissue samples were fixed and prepared as described in detail elsewhere (Brunk and Gustavii, 1973).

## RESULTS

Clear morphological and cytochemical differences were found between decidual cells at term and decidual cells from midpregnancy.

### *Decidual cells from midpregnancy*

These were morphologically well preserved and showed a distinct distribution of the enzyme reaction products (Figs. 1 and 2). Small necroses and focal infiltrates of polymorphonuclear leucocytes were occasionally noted.

### *Term decidual cells*

Although some of the term decidual cells were morphologically well-preserved, others showed distinct degenerative alterations, such as cytoplasmic vacuoles, nuclear pyknosis, and cellular shrinkage (Fig. 3). The normal rounded or polyhedral shape of these cells was thus lost, as well as their tile-like arrangement, and they appeared more or less spindle-shaped. Transitional forms ranging from cells of almost normal morphology to cells with advanced degenerative changes were seen. Focal necroses were less common in term decidua than in decidua of midpregnancy.

Cytochemical demonstration of acid phosphatase often showed diffuse cytoplasmic "staining" and sparse granular precipitates of the reaction products, indicating escape of enzymes from their initial lysosomal sites as a result of lysosome rupture or labilization, that is increased permeability of the lysosomal membranes (Fig. 4).

## DISCUSSION

The finding in the present study of degenerative alterations in decidual cells at term, before the onset of labour, is in agreement with observations made by other workers (Kaufman, 1896; Fujimura, 1921; Mossman, 1937; Wislocki, 1951). In previous reports, however, the decidual changes were only briefly noted, and no attempt was made to explain their possible clinical significance. Cytological alterations at the end of pregnancy have also been found in other tissues. For example, cellular changes, evidenced by vacuolated cytoplasm, and pyknotic or vesicular nuclei, have been observed in urinary smears obtained in late pregnancy (McCallin *et al.*, 1950). The extent of these changes increased as pregnancy advanced. A similar pattern has been found in vaginal smears (Lemberg-Siegfried and Stamm, 1955; Barnes and Zuspan, 1956; Lichtfus, 1959). Endometriotic tissue also undergoes alterations during pregnancy; first decidual transformation, followed later by degeneration, and finally necrosis and absorption (Kistner, 1960).

During the last few weeks of pregnancy, the levels of plasma oestrogens rise sharply (Klopper, 1973), while the progesterone output seems to fall (Turnbull *et al.*, 1974). These hormonal alterations are most likely to be responsible for the cellular changes observed in vaginal and urinary smears as well as in decidual cells. In men receiving high-dose oestrogen because of prostatic carcinoma, the urinary tract showed cytological changes similar to those in vaginal smears in late pregnancy (Papanicolaou, 1946). In addition, a withdrawal of progesterone is probably of major importance in the induction of these cellular changes, especially those in decidual cells, since progesterone is responsible for the decidualization of the oestrogen-primed endometrium (Reynolds, 1965).

Karim and Devlin (1967) have demonstrated high concentrations of prostaglandins in decidual tissue during labour. Since prostaglandins are potent myometrial stimulants and are used for the induction of labour and abortion, Karim (1968) has suggested that prostaglandins play a physiological role in parturition. This view is supported by the observation that aspirin, an inhibitor of prostaglandin synthetase (Vane, 1971), delayed the onset of spontaneous labour in humans (Lewis and Schulman, 1973) as well as of abortion by intra-amniotic injection of hypertonic saline (Waltman *et al.*, 1973). In rats, pregnancy was prolonged by an antibody specific for prostaglandins (Dunn *et al.*, 1973).

The release of a lysosomal marker enzyme observed in the present study is of interest since human decidua contains lysosomal (acid) phospholipase $A_2$ (Gustavii and Åkesson, unpublished observations). This enzyme specifically removes prostaglandin precursor acids, such as arachidonic acid, from the 2-position of phospholipids, which are a major constituent of cellular membranes (Van Den Bosch *et al.*, 1972). The observed leakage of lysosomal

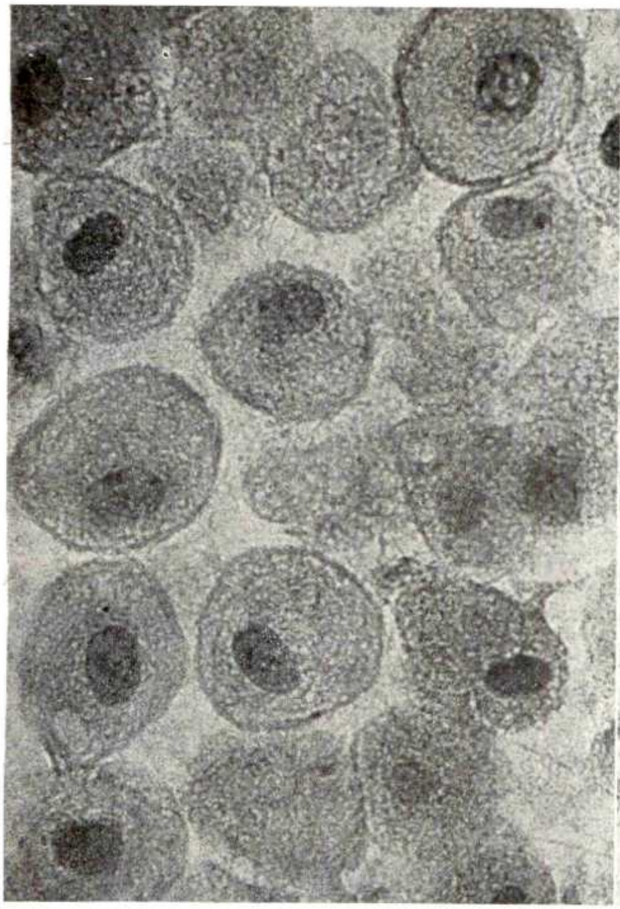

FIG. 1
Decidual cells from midpregnancy with well preserved morphology.
(Haematoxylin and eosin ×910)

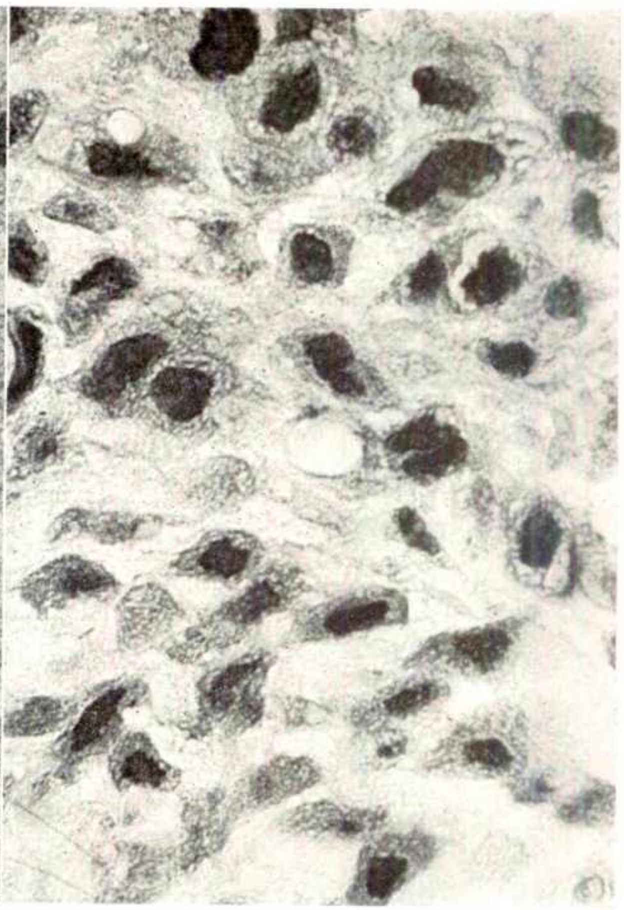

FIG. 3
Decidual cells from term pregnancy with cellular shrinkage, cytoplasmic vacuoles, and nuclear pyknosis.
(Haematoxylin and eosin ×910)

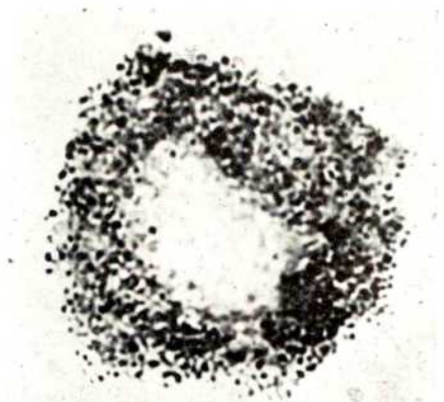

FIG. 2
Cytochemical demonstration of acid phosphatase in a decidual cell from midpregnancy showing granular distribution of reaction products.
(Incubation time 90 minutes ×1700)

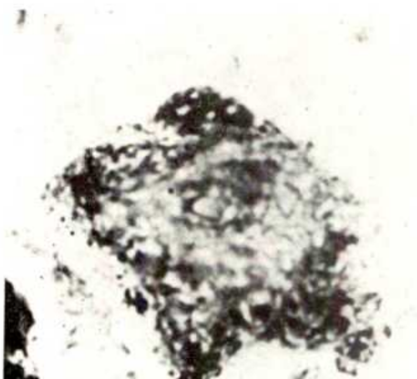

FIG. 4
Cytochemical demonstration of acid phosphatase in a decidual cell from term pregnancy showing diffuse cytoplasmic "staining", which indicates diffusion of hydrolytic enzymes from their initial lysosomal sites into the cytoplasm. Only a few granules can be seen.
(Incubation time 90 minutes ×1700)

enzymes within decidual cells is therefore probably followed by the synthesis and release of prostaglandins, leading in turn to increasing uterine activity and ultimately to the onset of labour.

There is strong evidence that lysosomes in the target organs of sex steroids are under hormonal control. Thus, the androgen-induced regression of the Müllerian ducts in male chick embryos is associated with an increase in the specific activity of several lysosomal enzymes (Scheib, 1963). Treatment of ovariectomized rats with oestrogen leads to labilization of the lysosomal membranes of specific target organs (Szego, 1972). In humans, the endometrium shows cyclic changes in lysosomal activity (Garcia-Bunuel and Brandes, 1966; Henzl *et al.*, 1972). It therefore seems probable that the observed release of lysosomal enzymes in term decidual cells is induced, at least in part, by oestrogens in high concentration. Since progesterone is responsible for the transformation of stroma cells into decidual cells in the oestrogen-primed endometrium, a withdrawal of this hormone might well have a labilizing influence on the membranes of decidual cells, including those of lysosomes, with the consequent release of lysosomal enzymes.

Since human decidual cells are exceedingly fragile and contain lysosomes of low resistance, they are especially susceptible to labilizing influences (Brunk and Gustavii, 1973). During labour, uterine contractions most probably have such a labilizing effect on decidual cells. It has been shown in pigs that lysosomes of liver cells are labilized *in vivo* by acidosis (Kallum, 1974),

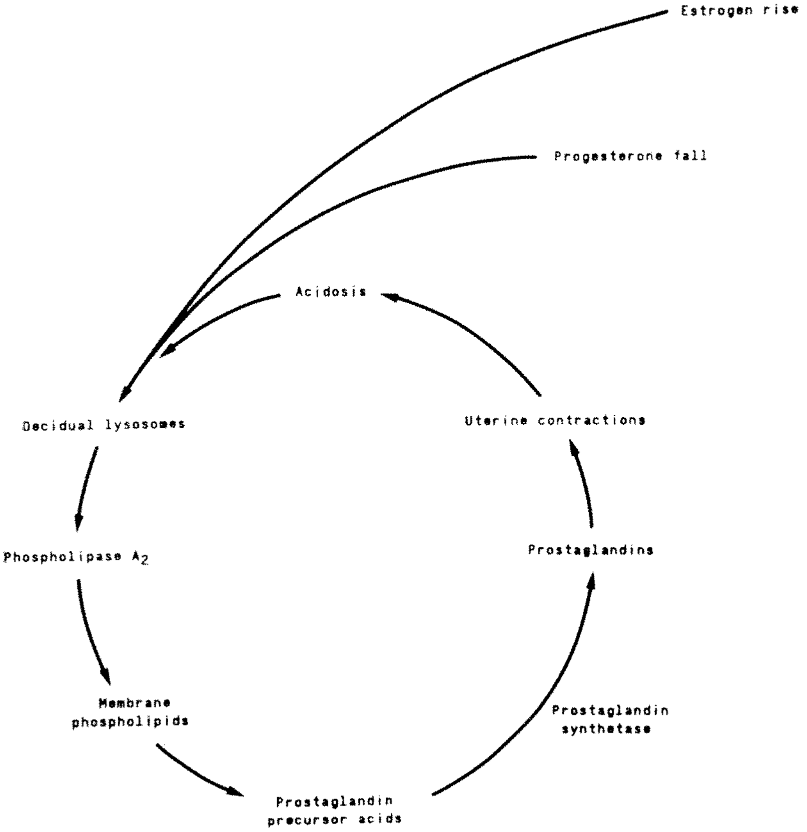

FIG. 5

Working hypothesis for the role played by lysosomal labilization in the mechanism triggering the onset of spontaneous labour at term. Only major factors are included.

a condition which normally occurs in the uterus during labour (Greiss and Andersson, 1968). Strong uterine contractions may also mechanically influence the fragile decidual cells. On the basis of the observations and assumptions mentioned above, the onset of labour may be explained as follows. The concentrations of prostaglandins in amniotic fluid rise progressively during the four weeks prior to labour (Hibbard *et al.*, 1974). It seems probable that the decidua is the source of these prostaglandins, and that their synthesis is triggered by the action of acid phospholipase $A_2$. With advancing gestation, this enzyme is probably liberated in increasing amounts from decidual lysosomes as a result of a progressive lysosomal labilization by sex steroids. As a consequence, the uterine contractions of pre-labour become progressively stronger and ultimately reach a point of no return. Thus, by causing acidosis in the uterus and thereby aggravating the labilization of the decidual lysosomes, the contractions of pre-labour finally become self-inducing. Massive amounts of lysosomal enzymes are then liberated, and large amounts of prostaglandins are synthesized and released. Uterine activity increases rapidly and labour has started. The model based on these concepts is presented in Figure 5, which is a simplified version of its predecessor (Gustavii, 1972). According to this scheme of reactions, released prostaglandins appear to be both the cause and the consequence of uterine contractions during labour.

## ACKNOWLEDGEMENTS

It is a pleasure to thank Mrs. Kerstin Sturesson and Mr. Lajos Erdős for technical assistance and the Ford Foundation for financial support.

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# THE AMNIOTIC FLUID FOAM TEST AND FAT CELL COUNT IN MALNOURISHED AND WELL-NOURISHED FETUSES

BY

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## Summary

A malnourished fetus has a higher concentration of pulmonary surfactant in the amniotic fluid than its well-nourished contemporary. The clinical value of this observation is discussed. Malnourished fetuses have higher "fat cell" counts in their amniotic fluid than well-nourished contemporaries. Well-nourished fetuses, at the same gestational age have high fat cell counts when the surfactant concentration is high, and low fat cell counts when the surfactant concentration is low. The relationship between surfactant concentration and the fat cell count is discussed.

OUR experience with the foam test described by Clements *et al.* (1972) and modified by Edwards and Baillie (1973), left the impression that the malnourished fetus had a higher concentration of pulmonary surfactant in its amniotic fluid than its well-nourished contemporary It is the purpose of this investigation to examine first the implied hypothesis that the malnourished fetus produces pulmonary surfactant earlier than its well-grown contemporary and second to examine the effect of fetal malnutrition upon the amniotic fluid "fat cell count" (Brosens and Gordon, 1966).

## PATIENTS

The subjects were 136 patients with singleton pregnancies who attended the antenatal clinic at Groote Schuur Hospital. Amniocentesis was performed in each pregnancy for a clinically acceptable reason, such as the clinical suspicion of growth retardation, rhesus isoimmunization, maternal disease likely to be benefited by induction of labour, prior to elective Caesarean section and in patients in preterm labour when the decision to delay or allow the labour to proceed was in doubt. Patients with severe rhesus disease, glucose intolerance or hydramnios were excluded.

All except six fetuses were delivered within five days of the amniocentesis. These six fetuses were all in the 30 to 35 week gestational age group and were all appropriately grown at delivery and presumably so at the time of amniocentesis.

## METHODS

### *Neonatal status*

*Nutrition.* A well-nourished newborn baby was defined as one whose weight at birth lay above the 10th percentile for its gestational age according to the standards of Lubchenko *et al.* (1972).

A malnourished baby was defined as one whose weight at birth lay below the 10th percentile for its gestational age using the same standards. Furthermore, any newborn baby in this group showing evidence of significant congenital abnormality or infection, particularly syphilis, was excluded, as were two babies born to mothers who had previously delivered similarly small babies which had subsequently done well (Ounsted and Ounsted, 1973). In this way it was hoped to obtain as pure a group of malnourished babies as possible.

*Gestational age.* This was assessed within two days of birth according to the criteria of Dubowitz *et al.* (1970) by one observer who did not know the results of amniocentesis. If there was a discrepancy of more than one week between this gestational age assessment and that suggested by the last menstrual period or a measurement of the biparietal diameter by ultrasound prior to 30 weeks then an independent Dubowitz assessment was taken. The mean of these two paediatric assessments was then taken to indicate the gestational age.

### *Amniotic fluid analysis*

Amniotic fluid was obtained by amniocentesis and subjected to immediate analysis for surfactant and fat cell scoring. Blood or meconium stained samples were discarded.

### *Surfactant (foam) scoring*

One ml. of amniotic fluid was mixed with 1 ml. of absolute alcohol in a scrupulously clean 10 mm. tube by vigorous shaking for 30 seconds. The top of the tube was covered by "parafilm" and after standing for 15 seconds the foam score was read according to the five point scale devised by Edwards and Baillie (1973).

### *Fat cell scoring*

Four drops of amniotic fluid were incubated with four drops of 0·1 per cent Nile blue sulphate at 37 °C. for three minutes. One drop of the mixture was examined microscopically and the fat cell score was recorded as the percentage of cells in the total squamous population associated with yellow staining globules.

## RESULTS

### *Foam scoring*

At the time of amniocentesis the fetuses were divided according to gestational age into three groups; 30 to 35 weeks, 36 to 37 weeks and 38 weeks or more. Figure 1 shows the distribution of foam scores, according to fetal nutrition in the three gestational age groups. It can be seen that with one exception the malnourished fetuses all possessed a foam score of 3 or 4.

TABLE I

*Foam score according to fetal nutrition and gestation*

| | Before 38 weeks | | After 38 weeks | |
|---|---|---|---|---|
| | Foam score | | Foam score | |
| | 0–2 | 3–4 | 0–2 | 3–4 |
| Number of malnourished fetuses | 1 | 15 | 0 | 9 |
| Number of well-nourished fetuses | 43 | 0 | 27 | 41 |

In Table I the fetuses were divided into those before and those after 38 weeks. The foam score was also divided into two groups 0 to 2 and 3 to 4.

Before 38 weeks of pregnancy a foam score of 3 to 4 was significantly associated with a malnourished fetus ($\chi^2 = 49$; $p<0{\cdot}001$). On the other hand a score of 0 to 2 excluded a malnourished fetus in all but one case. In this group, intrauterine malnutrition was undoubtedly associated with earlier development of surfactant.

After 38 weeks all the malnourished and the majority of the well-nourished fetuses had a foam score of 3 or 4 and a foam score of 3 or 4 in this group did not distinguish between the well-nourished and the malnourished fetus. This contrasts with the situation before 38 weeks.

### *Fat cell scoring*

The fetuses were divided into the same gestational age groups as for foam scoring, and the fat cell counts were divided into groups of 10 per cent intervals, 0 to 9, 10 to 19, etc. The distribution is shown in Figure 2. If the gestational age group 36 to 37 weeks is taken and the fetuses are divided according to their nutrition as before, and further as to whether more or less than 10 per cent of the amniotic fluid cells were "fat cells", it is clear that the malnourished group contained significantly more members with more than 10 per cent of their cells as fat cells than their well-nourished contemporaries (see Table

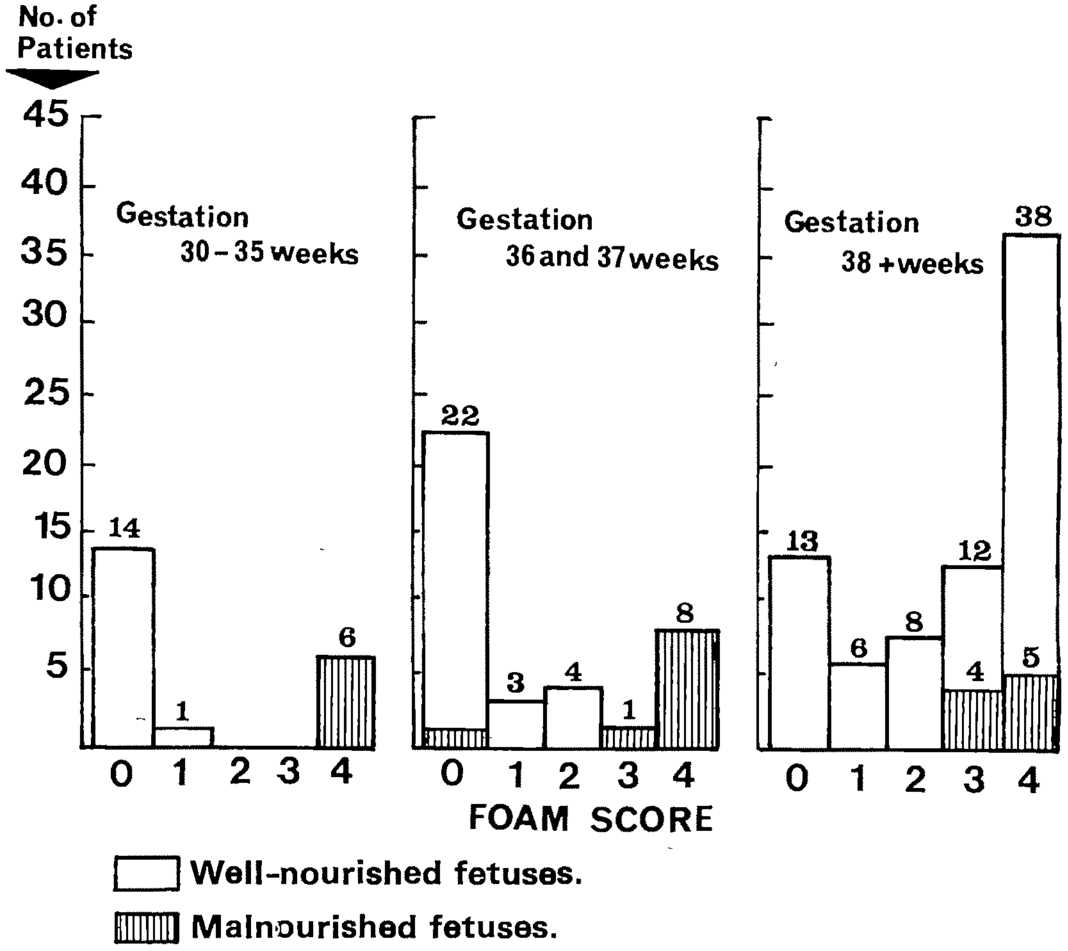

Fig. 1
Foam score at different gestational ages well-nourished and malnourished fetus.

TABLE II
*Fat cell score by fetal nutrition in the gestational age group 36–37 weeks*

| | Percentage of fat cells | |
|---|---|---|
| | 0–9 | 10+ |
| Number of malnourished fetuses | 2 | 8 |
| Number of well-nourished fetuses | 22 | 6 |

II; $p<0\cdot01$; reference Fourfold Table Test, Documenta Geigy Scientific Tables, 6th Edition, page 109).

### *The relationship between fat cell score and foam score in the well-nourished fetus*

Table III contrasts the fat cell score between two groups of well-nourished newborn in the gestational age group of 38 weeks and more.

TABLE III
*Fat cell score and foam score amongst well-nourished babies delivered at a gestational age of 38 weeks and more*

| Foam score | Number of fetuses | Fat cell score (per cent) | | Gestational age (weeks) | |
|---|---|---|---|---|---|
| | | Mean | SD | Mean | SD |
| 4 | 33 | 29·5 | 19·7 | 39·5 | 1·93 |
| 0 | 13 | 9·3 | 10·6 | 39·2 | 2·36 |

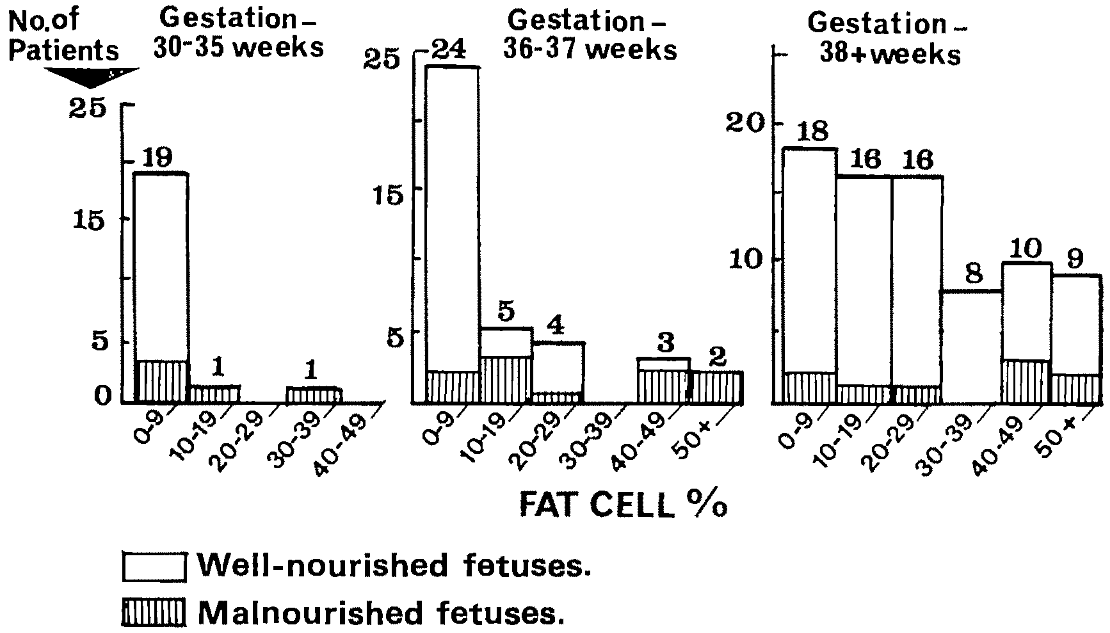

FIG. 2

Fat cell scores by gestational age groups in well-nourished and malnourished fetuses.

The two groups consisted of those with a foam score of 0 and those with a foam score of 4.

The group with a foam score of 4 had a significantly higher fat cell score than those in the group with a foam score of 0. ($t = 3{\cdot}48$; $p < 0{\cdot}01$). The mean gestational ages of the fetuses in the two groups were not significantly different.

## DISCUSSION

One purpose of this investigation was to examine whether a malnourished fetus produced pulmonary surfactant earlier than a well-nourished fetus. The results have shown that the group of small for gestational age fetuses, defined so as to include as pure a sample of malnourished fetuses as possible, did have the two maximal foam scores (with one exception), provided that the amniotic fluid was obtained within five days of delivery. On the other hand these scores were never obtained in a well-nourished fetus prior to 38 weeks, but were common after that time. Since the foam score, although simple, is an index of pulmonary surfactant it is right to conclude that a malnourished fetus produces pulmonary surfactant earlier than its well-grown contemporary.

This conclusion has two important clinical applications, and one limitation. In the first place it would appear that a high foam score (3 or 4) prior to 38 weeks is diagnostic of intra-uterine malnutrition while a low foam score would not exclude such a diagnosis. In several patients who had serial amniocenteses the foam score rose from 0 to 4 over a period of one week; the actual rate of rise may have been even more rapid. This may well be why we did find one malnourished fetus with a foam score of 0. Serial amniocentesis would therefore be required to take account of this phenomenon and the need for serial amniocentesis is a serious limitation to the application of principle to practice.

Although a malnourished fetus with a high foam score would have mature lungs it does not mean that it should be delivered.

Follow-up studies have shown that severely malnourished fetuses have a poor prognosis (Davies and Davis, 1970; Drillien, 1973). It is not certain at what level of malnutrition the irreversible changes in the fetus take place. The appearance of an inappropriately high concentration of surfactant for gestational age in malnourished fetuses can be regarded as a fetal response to its own malnutrution. It is important, therefore, to be aware of the temporal relation of this fetal response to the onset of the irreversible fetal growth changes which might prejudice the future for the child. If the appearance of surfactant is an early response to malnutrition, then the phenomenon would appear to be a potentially valuable diagnostic aid. On the other hand if the response is late, then its value is reduced.

Another purpose of this investigation was to examine the relationship of fetal nutrition to the "fat cell" count in amniotic fluid. At a gestational age of 36 to 37 weeks, the malnourished fetus had a higher foam score and also more fat cells than the well-nourished fetus. After 38 weeks, well-nourished fetuses have a high fat cell count when the foam score is high and a low fat cell count when the foam score is low. Thus it would seem that the fat cell count is not determined by gestational age alone, and that the fat cell count and foam score are related variables. It is not surprising, therefore, that gestational age cannot be predicted accurately from the fat cell count.

## ACKNOWLEDGEMENTS

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# A COMPARATIVE CLINICAL EVALUATION OF THE FOAM TEST AND PHOSPHOLIPID ASSAY OF AMNIOTIC FLUID

BY

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## Summary

Amniotic fluid taken by amniocentesis from patients at varying stages of gestation was analyzed for surfactant by the foam test, total phospholipid phosphorus concentration, and alkali labile phospholipid phosphorus concentration. The total and alkali labile phospholipid concentrations increased, and the ratio of alkali labile to total phospholipid concentration fell slightly as gestational age increased. The mean total and alkali labile phospholipid phosphorus concentrations increased with the foam test score, but there was considerable overlap in the phospholipid concentrations between the different groups of foam test results. In our experience only when the foam test is negative does hyaline membrane disease occur, and even then not invariably. Only when the foam test is negative, therefore, is further analysis of amniotic fluid for surfactant indicated. The simplest procedure is then to measure the amniotic fluid total phospholipid concentration. This will resolve the doubt as to whether the newborn is liable to hyaline membrane disease in about one half of the cases. The suggestions by other workers that the ratio between alkali labile phospholipid and total phospholipid would resolve this doubt has not been confirmed by our study.

MOST observers are agreed that examination of the amniotic fluid can provide an index of fetal pulmonary surfactant but, because surfactant is so far unidentified, which of the many measurements in current use is best related to it remains uncertain.

Lecithin is certainly not established in the role of pulmonary surfactant, because its relationship to the latter is inconstant (Broughton *et al.*, 1970; Platzker *et al.*, 1971). It is questionable therefore whether the considerable effort of measuring lecithin (Gluck *et al.* 1971; Bhagwanani *et al.*, 1972) is worthwhile, especially when there are much simpler ways of assessing surfactant in amniotic fluid.

Simple physical measurements of surfactant, the foam test, have been described by Clements *et al.* (1972) and Edwards and Baillie (1973) and a simple biochemical measurement by Bayer *et al.* (1973). This last group described a method of measuring the total and alkali labile phospholipid phosphorus concentrations in amniotic fluid, and presented evidence which suggested that the ratio between them was a significant index of fetal pulmonary surfactant.

It is the purpose of this presentation firstly to report on the relation between the foam score and the phospholipid concentrations and to examine the extent to which they are complementary in clinical practice; secondly, to test the suggestion that the ratio between alkali labile and total phospholipid phosphorus concentrations in amniotic fluid is an index of fetal pulmonary surfactant.

## METHODS

Amniotic fluid uncontaminated by meconium or blood was obtained at amniocentesis from 111 singleton pregnancies, taken from all racial groups, either prior to induction of labour, before Caesarean section, or in connection with Rhesus disease or for the investigation of possible fetal abnormality. Pregnancies complicated by hydramnios or diabetes were excluded.

### *Amniotic fluid analysis*

*Phospholipid determination.* The concentration of total and alkali labile phospholipid phosphorus was measured by the method described by Bayer *et al.* (1973). Each analysis was performed immediately and in duplicate.

*Foam score.* The foam test was performed in duplicate as described by Edwards and Baillie (1973) and scored on a five point scale from 0 to 4. The expression foam test has been used throughout this article. The test has variously been described as the "shake test" or the "bubble test", but it is felt that because its endpoint involves an assessment of foam, the term "foam test" is more descriptive.

### *Gestational ageing*

The gestational age of the fetus at the time of amniocentesis was calculated from the paediatric age assessment of the fetus at birth according to Dubowitz *et al.* (1970). When this corresponded with prediction from the date of the last menstrual period, or an ultrasonic measurement of biparietal diameter performed prior to 30 weeks it was accepted. Otherwise the mean of two paediatric scores obtained by independent observers was taken as the gestational age at birth.

The newborn were separated into the appropriately grown for gestational age (AGA) and the small for gestational age (SGA) at birth, according to their position in relation to the 10th percentile on the standard charts of Lubchenko *et al.* (1972).

## RESULTS

### *Total and alkali phospholipids and their ratio as a function of gestational age in the fetus appropriately grown at delivery*

Table I describes the mean levels, their ranges and standard deviations for 76 appropriately

TABLE I

*The concentration in amniotic fluid of total phospholipid, alkali labile phospholipid and their ratio, as a function of gestational age in appropriately grown fetuses*

| Gestational age weeks | Number | Total phospholipid (μg. of P/ml.) | | | Alkali labile phospholipid (μg. of P/ml.) | | | Ratio alkali labile / Total phospholipid | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | S.D. | Range | Mean | S.D. | Range | Mean | S.D. | Range |
| Below 26 | 3 | 1·21 | 0·26 | 1·0<br>1·5 | 1·4 | 0·36 | 1·0<br>1·7 | 1·16 | 0·28 | 1·00<br>1·49 |
| 26–29 | 3 | 1·34 | 0·15 | 1·2<br>1·5 | 1·18 | 0·11 | 1·1<br>1·3 | 0·88 | 0·03 | 0·86<br>0·92 |
| Group (A)<br>30–33 | 9 | 1·70 | 0·54 | 1·2<br>2·7 | 1·71 | 0·60 | 1·1<br>2·8 | 1·03 | 0·18 | 0·79<br>1·42 |
| Group (B)<br>34–37 | 26 | 4·50 | 2·47 | 1·2<br>11·2 | 3·83 | 2·21 | 1·1<br>9·8 | 0·87 | 0·24 | 0·40<br>1·36 |
| Group (C)<br>38+ | 35 | 9·37 | 3·30 | 5·1<br>17·4 | 7·12 | 2·19 | 3·6<br>11·4 | 0·78 | 0·14 | 0·46<br>1·06 |

grown fetuses divided into five gestational age groups.

It can be seen that the total and alkali labile phospholipids increased steadily through the three gestational age groups: 30 to 33 weeks (A), 34 to 37 weeks (B), and the 38 and more weeks (C). The ratio of alkali labile to total phospholipid changed little and even declined slightly. The differences between the groups are shown by Table II where the "t" test has been applied.

Table II shows that there is no significant difference in the ratio of alkali labile to total phospholipid between the groups taken sequentially, but that a significant difference exists between the group A (30 to 33 weeks) and group C (38 weeks and more), where the mean ratio actually falls.

Inspection of the alkali to total phospholipid ratio column in Table I shows that in each of the three gestational age groups A, B and C, the alkali labile phospholipid measurement exceeded the total phospholipid at some time. This was particularly evident in group A, where the mean values of total and alkali labile phospholipid were the lowest of the three groups. At no time prior to 34 weeks did the ratio fall below 0·65 which Bayer *et al.* (1973) considered was the lowest level to indicate freedom from the risk of hyaline membrane disease.

### *The total and alkali labile phospholipids and their ratio in malnourished fetuses*

There were five malnourished fetuses in this sample. Four were in the gestational age group 34 to 37 weeks (B) and one in the group 30 to 33 weeks (A). The phospholipid levels of the four in group B are presented separately in Table III in order to compare them with their appropriately grown contemporaries.

TABLE II

*Significance of the differences in mean total phospholipid, alkali labile phospholipid and their ratio between gestational age groups*

| Gestational age | Total phospholipid | | Alkali labile phospholipid | | Ratio alkali labile total phospholipid groups | |
|---|---|---|---|---|---|---|
| comparison | "t" | P | "t" | P | "t" | P |
| A/B | 5·4* | <0·001 | 4·4* | <0·001 | 1·82 | NS |
| B/C | 6·3 | <0·001 | 7·3 | <0·001 | 1·84 | NS |
| A/C | 13·1* | <0·001 | 12·9* | <0·001 | 4·51 | <0·001 |

NS = Not significant.

*Due to unequal variances, modified values of "t" and "degrees of freedom" have been used according to the method of Dixon and Massey (1967).

TABLE III

*A comparison of phospholipid levels between malnourished and well-nourished fetuses in the gestational age groups 34 to 37 weeks (B)*

| | Number | Total phospholipid (μg. of P/ml.) | | Alkali labile phospholipid (μg. of P/ml.) | | Ratio alkali labile / Total phospholipid | |
|---|---|---|---|---|---|---|---|
| | | Mean | S.D. | Mean | S.D. | Mean | S.D. |
| Well-nourished | 26 | 4·50 | 2·47 | 3·83 | 2·21 | 0·87 | 0·24 |
| Malnourished | 4 | 15·00 | 4·33 | 12·30 | 3·38 | 0·83 | 0·065 |

The mean total phospholipid concentration ("t"= 7·16 p<0·001) and alkali labile phospholipid concentration ("t"= 6·67 p<0·001) were significantly higher in malnourished than in well-nourished fetuses in this group. The ratios were not significantly different. The gestational ages of the four malnourished newborn were 37, 36, 36, and 35 weeks respectively in comparison with a mean of 36 weeks for the well-nourished babies in this group.

The malnourished newborn had significantly higher mean total and alkali labile phospholipid concentrations than the well nourished fetuses, but the mean ratio of alkali labile to total phospholipid was not significantly different.

### *Comparison between the foam score, total phospholipid and alkali labile phospholipid phosphorus concentration*

This comparison is shown in Figures 1 and 2. The foam scores are separated into five groups

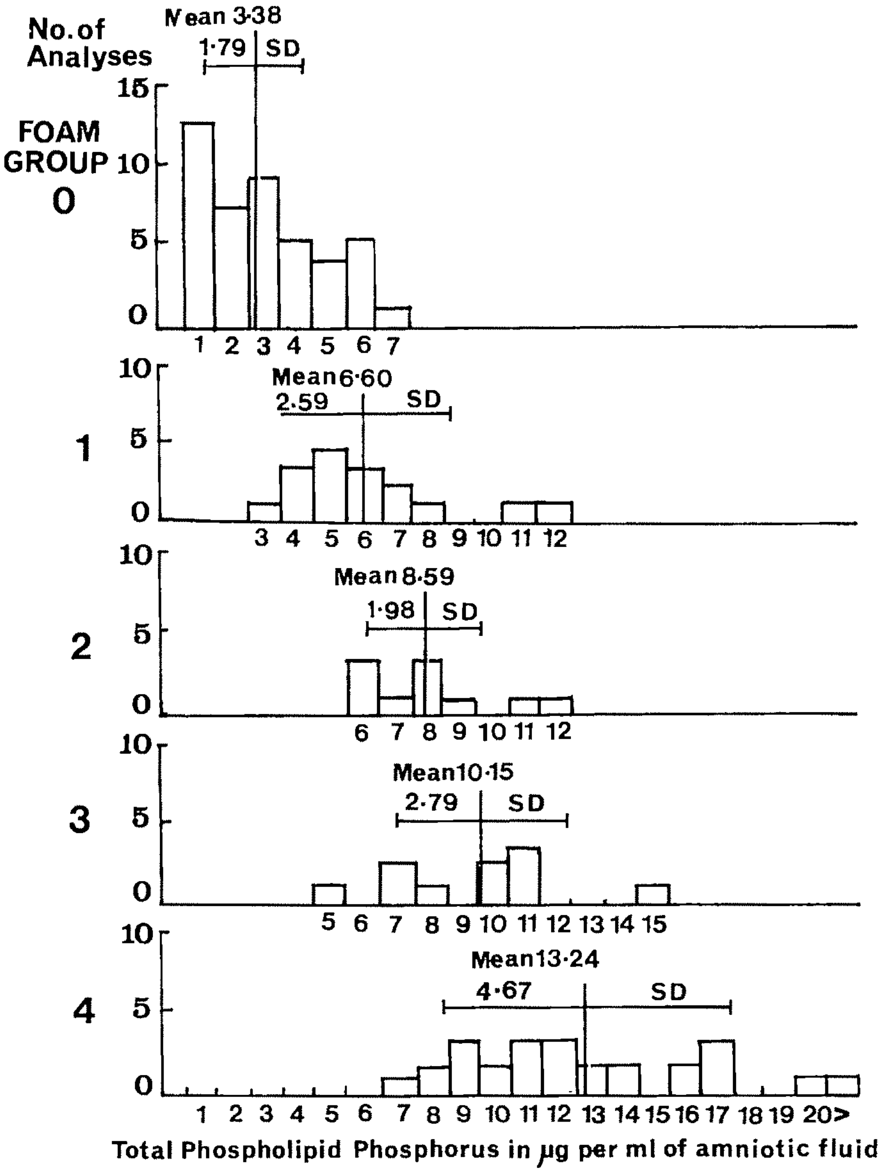

FIG. 1
The relationship between total phospholipid phosphorus and the foam score.

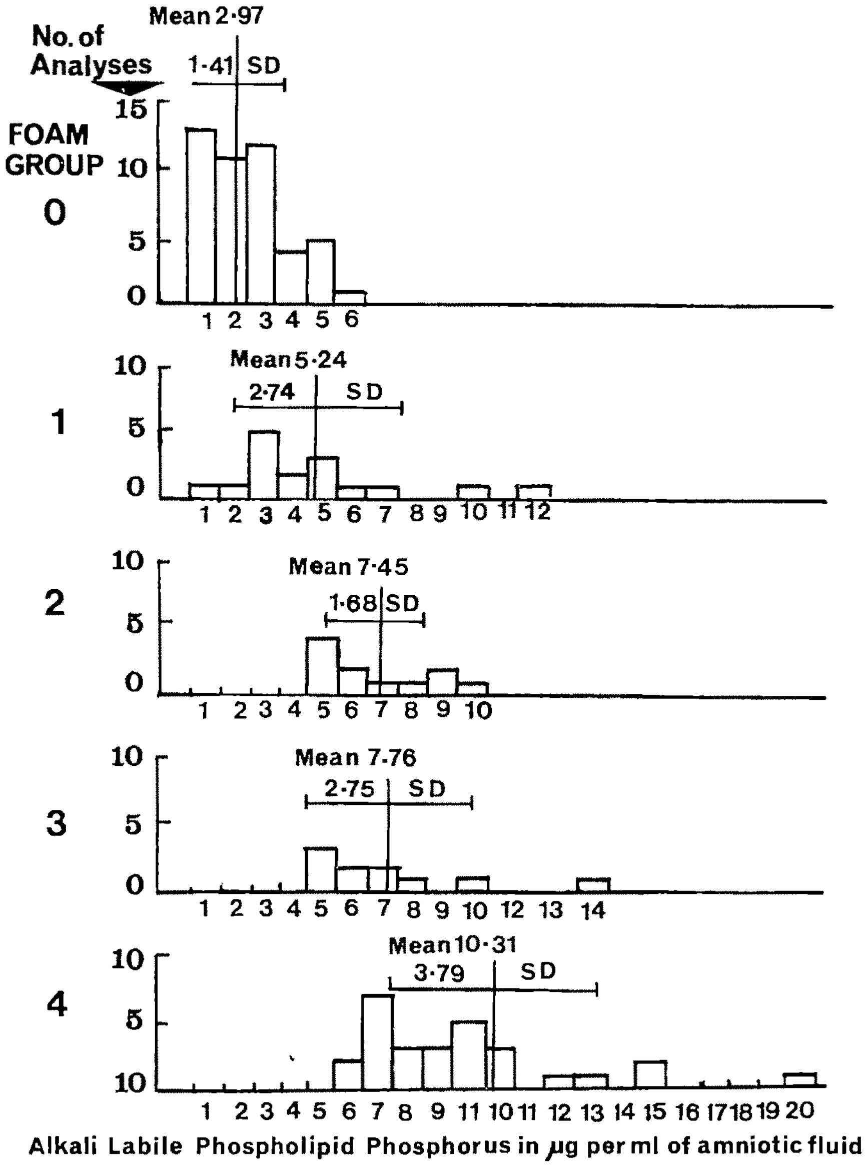

FIG. 2
The relationship between alkali labile phospholipid phosphorus and the foam score.

(0 to 4) and histograms have been constructed showing the phospholipid phosphorus associated with each group. The phospholipid phosphorus results have been presented in blocks of 1 μg. per ml. (equivalent to 2·5 mg. phospholipid per 100 ml. assuming a 4 per cent phosphorus content of phospholipid). The principle feature of note in these illustrations is the overlap between foam groups in both the total and alkali labile phospholipid phosphorus results. The lowest total phospholipid phosphorus concentration associated with a foam score of 1 was 3·5 μg. per ml.

## DISCUSSION

In the original report by Edwards and Baillie

(1973) hyaline membrane disease was not found in association with a foam score of 1, and very often not found when the foam score was zero. This observation has been amply confirmed by our unpublished subsequent experience. Whatever the foam test measures, a positive result gives assurance that the fetus, however delivered, will not develop hyaline membrane disease. When the foam test is negative there is no such assurance, hence the need to distinguish in this group those fetuses which are at risk of developing hyaline membrane disease. It is tempting to suggest that measurement of the total phospholipid phosphorus in amniotic fluid would achieve this. Our results have shown that approximately one-half of the samples with a foam score of 0 had a total phospholipid in the range associated with a positive foam score in other specimens. It is suggested that in these specimens there would be sufficient surfactant to predict freedom from hyaline membrane disease. On the basis of this empirical argument, the total phospholipid phosphorus concentration would help to resolve some of the doubt left as a result of the simple foam test. The merit of performing a total phospholipid assay as opposed to any other biochemical phospholipid assay is that it is rapid, reproducible and can be readily performed by careful but otherwise unskilled staff within two hours.

Our results have shown that alkali labile and total phospholipid concentrations increase with gestational age, thus confirming the observation of Bayer *et al.* (1973), but we have been unable to confirm their observation that the ratio between these two fractions increases with gestational age; in fact we have found that it declines. If their observation that a ratio of 0·65 between alkali labile and total phospholipid confers freedom from hyaline membrane disease is true, then none of our fetuses prior to 34 weeks would have developed hyaline membrane disease. Since this sample of 15 contained 6 below 30 weeks we consider this inherently unlikely although none were delivered so early so the point must remain unproved. There might well be technical reasons for our failure to confirm their results, and if so then there is all the more reason for not wishing to place reliance on this ratio in clinical practice.

A test of pulmonary surfactant requires to be most discriminating when the levels are low. If technical factors are responsible for errors in measurement then these are relatively magnified when the levels are themselves low and further magnified by the use of a ratio. In our practice we have found no place for the use of this ratio.

We have been able to confirm that the malnourished fetus produces phospholipid earlier than its well nourished contemporary. This is an observation of considerable significance in terms of diagnosis and management of such fetuses (Fairbrother *et al.,* 1975). We suggest that the first analysis of amniotic fluid for pulmonary surfactant should be performed by a simple foam test, and only if this is negative should further analysis be undertaken. Of the biochemical methods at present available, determination of the total phospholipid phosphorus is the simplest. All approaches to measuring pulmonary surfactant are empirical and simplicity is a virtue until pulmonary surfactant is identified. Determination of the ratio between alkali labile and total phospholipid involves complicated biochemical procedures which in our hands have given results which would have deluded us in our clinical management.

## ACKNOWLEDGEMENTS

We thank Miss B. Becket, Medical Artist, and Miss C. Vader, Statistician, both of the Department of Obstetrics and Gynaecology for their help in the preparation of this work.

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# PALMITIC ACID AND LECITHIN MEASUREMENTS IN AMNIOTIC FLUID

BY

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## Summary

A method is described for the rapid and quantitative estimation of total amniotic fluid palmitic acid. Palmitic acid and lecithin were measured in 140 samples of amniotic fluid in normal and abnormal pregnancy, and the correlation coefficient between the two parameters was 0·93. It is concluded that amniotic fluid palmitic acid measurements are of value in the prenatal determination of fetal pulmonary maturity.

THE risk that the infant might develop the respiratory distress syndrome (RDS) after delivery is an important factor in the timing of induction of labour or of elective Caesarean section. RDS is associated with a deficiency of pulmonary surfactant (Pattle, 1965; Scopes, 1971) and the finding that phospholipids from the fetal lung gain access to the amniotic fluid (Nelson, 1960) has led to the use of amniotic fluid phospholipid analysis for the assessment of fetal pulmonary maturity.

There is no agreement at the present time as to the best analytical procedure. The ratio of lecithin to sphingomyelin has been determined chromatographically in the method introduced by Gluck *et al.* (1971). This method is semi-quantitative but is sensitive to small variations in technique (Gebhardt and Dubbeldam, 1973), and the chromatograms are difficult to interpret in borderline cases. The usefulness of the method has been confirmed by some workers (Whitfield *et al.*, 1972; Hobbins *et al.*, 1972; Bryson *et al.*, 1972) but not by others (Arvidson *et al.*, 1972; Nelson, 1972; Bhagwanani *et al.*, 1972).

Bhagwanani *et al.* (1972) measured the concentration of lecithin in amniotic fluid, and this has been confirmed by Phizackerley and Moore (unpublished observations), while Bayer *et al.* (1973) estimated lecithin as a proportion of amniotic fluid phospholipids. Both methods depend on a phosphate estimation and are time consuming, though quantitative.

This paper proposes a rapid and quantitative estimation of amniotic fluid lecithin by means of the palmitic acid moiety rather than the phosphate moiety. Warren *et al.* (1973 and 1974*a*) have shown that total amniotic fluid palmitic acid correlates well with the L/S ratio determined chromatographically, and a better correlation with total lecithin concentration is described here.

Both methods rely upon the high palmitic acid content of lecithin isolated from amniotic fluid (P. J. R. Phizackerley, personal communication) and pulmonary surfactant (Frosolono *et al.*, 1970), and that lecithin is the major contributor of palmitic acid to the amniotic fluid (Warren *et al.*, 1974*b*).

## METHODS

### *Estimation of palmitic acid*

One ml. of well mixed uncentrifuged amniotic fluid was extracted with four ml. of chloroform/methanol 2 : 1 (v/v) (Folch *et al.*, 1957) after the addition of 0·1 ml. of a 300 μg./ml. solution of heptadecanoic acid in methanol. The mixture was shaken vigorously for two minutes and the phases separated by centrifuging on a bench centrifuge for five minutes. The organic phase was transferred to a clean test tube and the contents evaporated to dryness under a stream of nitrogen at 50 °C.

The lipid was subjected to hydrolysis in 0·5 ml. of freshly prepared 0·025M NaOH in 90 per cent methanol at 37 °C. for 20 minutes (Dawson, 1960). The mix was acidified by addition of one ml. of normal HCl and the fatty acids extracted twice with 2 ml. of diethyl ether.

The ether phases were combined in a clean tube and 0·5 ml. of methanol was added followed by drops of diazomethane in ether until a yellow colour was retained (Schlenk and Gellerman,

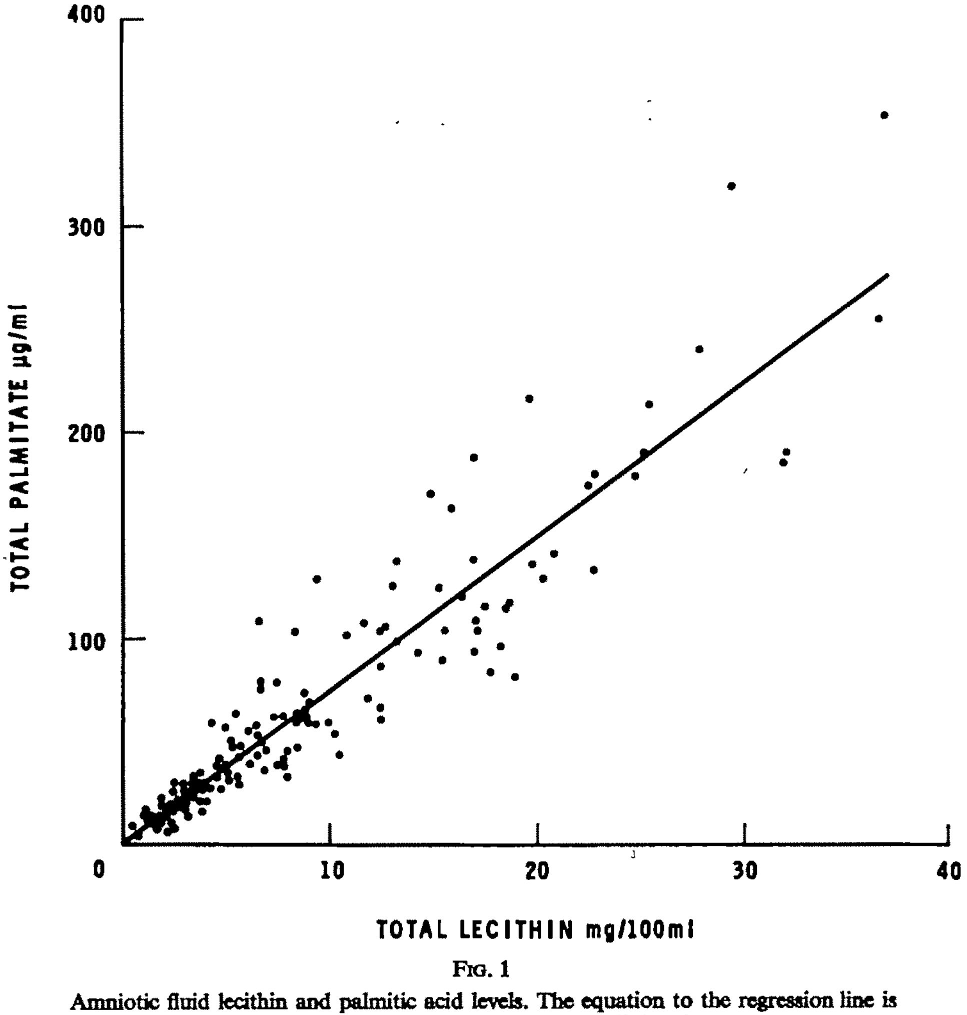

FIG. 1

Amniotic fluid lecithin and palmitic acid levels. The equation to the regression line is $y = 7{\cdot}53x - 0{\cdot}88$, and the correlation coefficient is 0·93.

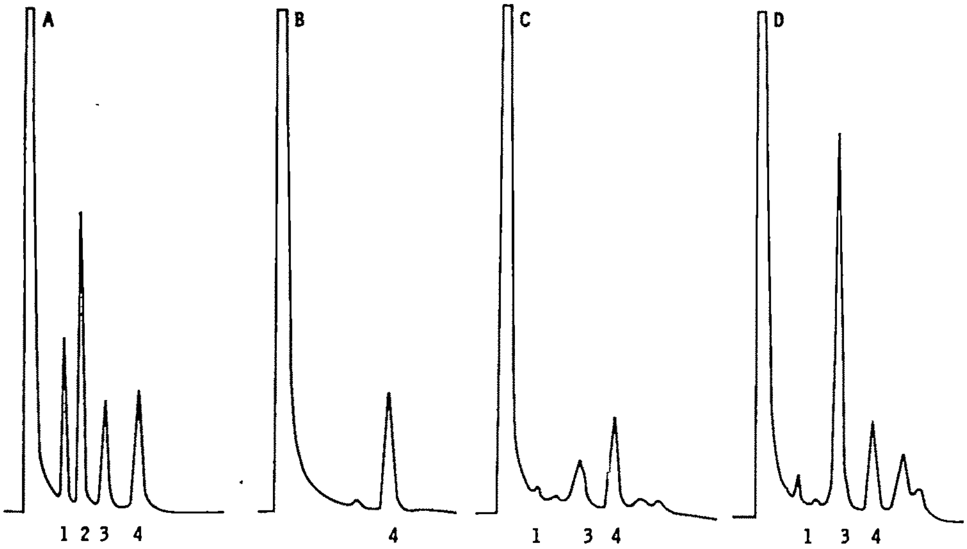

FIG. 2
Typical gas chromatograph recordings of standard (A), saline blank (B), low palmitate sample (C), and high palmitate sample (D). The peaks are myristic acid (1), pentadecanoic acid (2), palmitic acid (3), and heptadecanoic acid (4).

1960). After standing at room temperature for ten minutes the solution was evaporated to dryness at room temperature and the residue dissolved in 50 μl. of hexane.

Three μl. were used for chromatography on a five foot column of Apiezon L, 10 per cent on supasorb AW-HMDS (80–100 mesh), run at 270 °C. on a Hewlett Packard model 402 gas chromatograph equipped with a flame ionization detector. Nitrogen was used as carrier gas and both injection port and detector were maintained at 270 °C.

*Estimation of lecithin*

The method of Bayer *et al.* (1973) was used, and results for alkali labile phospholipid were expressed as mg./100 ml. of amniotic fluid, assuming that dipalmitoyl lecithin was the major alkali-labile phospholipid.

*Collection and storage of samples*

A total of 140 specimens of amniotic fluid was obtained by amniocentesis, amniotomy for induction, Caesarean section, and at termination of early pregnancy. Samples contaminated by blood or meconium were discarded. Analysis was carried out within 48 hours of collection, or the samples were stored frozen until analysis.

## RESULTS

The results of palmitic acid and lecithin analyses on the 140 samples collected are shown in Figure 1. There was a good linear correlation between the two parameters, the equation to the regression line being $y = 7{\cdot}53x - 0{\cdot}88$, and the correlation coefficient being 0·93. Typical gas chromatograph recordings of the fatty acid methyl esters are shown in Figure 2.

The recovery of lecithin added to amniotic fluid was high (Fig. 3), and both lecithin isolated from amniotic fluid and an authentic lecithin sample were completely hydrolysed by methanolic alkali under the conditions described (Fig. 4). Sphingomyelin was unaffected by this treatment. Liberated fatty acids were completely extracted from acid solution by ether (Table I). The precision of the method was estimated by analysing 53 samples in duplicate. The differences between the duplicates in three ranges of palmitic acid concentration are shown in Table II. Duplication was found to be good at levels below 40 μg./ml.

Assays of serial samples from the same patient were made in several cases, and these were characterized by sharp increases in palmitic acid concentrations (Fig. 5). None of these infants suffered any complications after delivery.

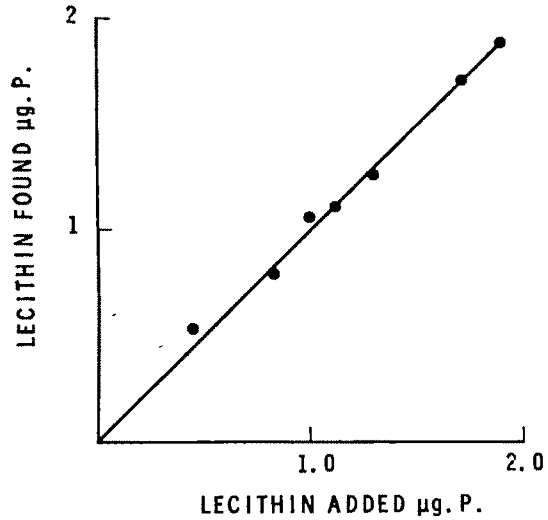

FIG. 3

The recovery of lecithin added to amniotic fluid.

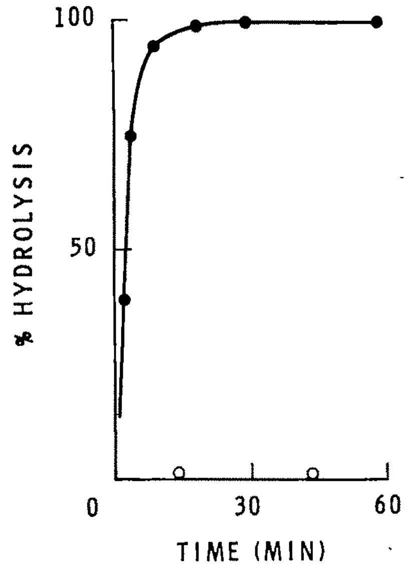

FIG. 4

Time course of hydrolysis of lecithin (●) and sphingomyelin (○).

TABLE I

*Extraction of fatty acids from aqueous acid solution. About 0·05 uCi U-$^{14}$C-palmitic acid was added to hydrolyzed amniotic fluid lipids which were then extracted twice with 2 ml. of solvent*

| Solvent | $^{14}$C-palmitate added (c.p.m.) | $^{14}$C-palmitate recovered (c.p.m.) | Percentage recovered |
|---|---|---|---|
| Diethyl ether | 145065 | 144737 | 99·8 |
| Chloroform | 117555 | 117135 | 99·6 |
| Petroleum spirit (40°–60°) | 133503 | 100705 | 75·4 |

TABLE II

*Precision of duplicate estimations in several ranges of palmitic acid concentration*

| Palmitic acid concentration range (µg./ml.) | No. of samples assayed in duplicate | Mean difference (±SD)* between duplicates (µg./ml.) |
|---|---|---|
| 0–20 | 18 | 1·2 ± 0·9 |
| 21–40 | 22 | 2·8 ± 2·6 |
| Above 40 | 13 | 9·0 ± 7·8 |

* SD = Standard Deviation.

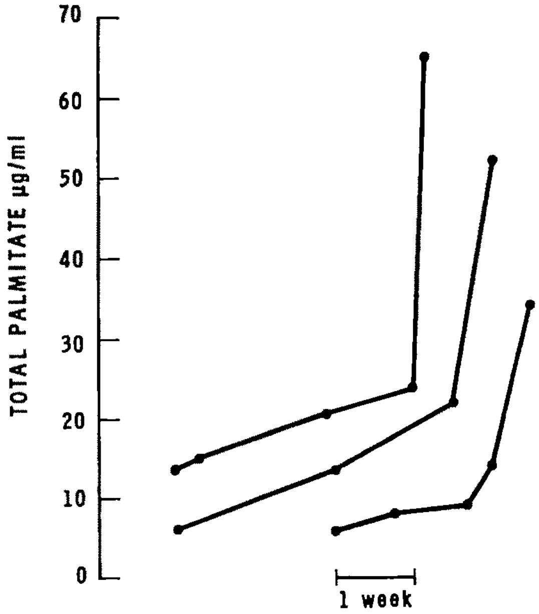

FIG. 5

Serial palmitic acid estimations on three patients in late pregnancy.

## DISCUSSION

It is now acknowledged that amniotic fluid phospholipid estimations are of major importance in the prediction of fetal pulmonary maturity, though the ideal method of analysis remains to be established. Thin layer chromatography can be fairly rapid but is at best only semi-quantitative. Chemical methods for estimating lecithin phosphate have proved to be too laborious for routine use.

The analysis of amniotic fluid lecithin through the palmitic acid moiety is both quantitative and rapid, as a sample can be analysed in about one hour. The addition of an internal standard at the outset of the procedure eliminates several sources of error, and the comparative ease of the technique allows for an element of quality control to be introduced by the use of refrigerated pooled amniotic fluid.

Bhagwanani *et al.* (1972) suggested that a lecithin concentration greater than 3·5 mg./100 ml. indicated maturity, and Phizackerley and Moore (unpublished data) failed to observe RDS in infants in which the lecithin concentration was greater than 4·0 mg./100 ml. up to 48 hours before delivery. The data in Figure 1 would therefore indicate that RDS should be absent in infants delivered with amniotic fluid palmitic acid levels greater than 25 μg./ml. This is in accord with the data of Warren *et al.* (1973*a* and *b*) in which a level of 19 μg./ml. was the lowest mature level found.

The maximum rate of increase in palmitic acid concentration seen in Figure 5 was 12 μg./ml./day, meaning that the onset of maturity is rapid. This emphasizes similar observations by Bhagwanani *et al.* (1972) and Bayer *et al.* (1973), and thus a low result from a sample taken more than two days before a delivery in which RDS was not a complicating factor does not invalidate the method.

A fuller clinical evaluation of amniotic fluid palmitic acid measurements is published elsewhere (MacLennan *et al.*, 1974).

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# PALMITIC ACID LEVELS IN AMNIOTIC FLUID AND THE SHAKE TEST

BY

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## Summary

One hundred and six samples of amniotic fluid were analyzed independently by four clinicians for surfactant content, using the shake test and the palmitic acid content of the amniotic fluid. The variation of the results in the 424 shake tests were examined and the shake tests correlated with the palmitic acid levels. A positive shake test to a dilution of 1 : 4 had a 100 per cent correlation with a mature palmitic acid level over 35 μg./ml., while a positive test at a dilution 1 : 2 gave a 5·6 per cent false positive rate. A high false negative rate was found, however, and estimates of the chance of a mature surfactant level in relation to the range of shake tests results are given. Eleven patients had low palmitic acid levels in the amniotic fluid prior to delivery and three of the infants developed the respiratory distress syndrome.

THE prediction of fetal lung maturity by the estimation of amniotic fluid phospholipid levels to reduce the risk of respiratory distress syndrome in the newborn is now established (Gluck *et al.*, 1971; Bhagwanani *et al.*, 1972; Whitfield *et al.*, 1972; Bayer *et al.*, 1973). The shake test or bubble stability test (Clements *et al.*, 1972) is now widely used as a preliminary screening test and also in hospitals not able to undertake the biochemical tests. A positive shake test has been shown to correlate well with the lecithin/sphyngomyelin ratio and the clinical outcome (Boehm *et al.*, 1973; Parkinson and Harvey, 1973; Dewhurst *et al.*, 1973; Sheppard *et al.*, 1974). A high incidence of false negative results has been shown in comparative studies of the shake test and lecithin concentration in the amniotic fluid (Bhagwanani *et al.*, 1973; Fisher *et al.*, 1973). In view of the widespread use of this simple test we have studied the variation in the results of the shake test when carried out by obstetric staff as a side room procedure. The risks of acting on the shake test result alone for a given dilution to which the test is positive have also been assessed.

In this study the results of the shake test have been correlated with amniotic fluid palmitic acid levels. Ninety per cent of the total palmitic acid in the amniotic fluid originates from lecithin (Warren *et al.*, 1974). A clear correlation between amniotic fluid palmitic acid levels, the lecithin/sphyngomyelin ratio and the incidence of respiratory distress syndrome in the infant has been reported (Warren *et al.*, 1974; Ekelund *et al.*, 1973).

## METHODS

Amniotic fluid was obtained at amniotomy with a Drew-Smythe catheter or by trans-abdominal amniocentesis. Specimens containing blood or meconium were excluded and uncontaminated samples from 106 patients were studied. Amniotic fluid samples were obtained from pregnancies ranging from 30 to 41 weeks gestation. The specimen was mixed and divided into 2 aliquots, one for palmitic acid estimation which was carried out without delay and one for the shake tests.

The shake test was performed independently by four clinicians (two registrars and two senior house officers). All four tests were performed on the same sample within 12 hours of the sample being obtained. Each investigator followed the method in detail and the results were not compared until all 106 samples had been tested. The palmitic acid levels were used in the clinical management of each patient.

Clean disposable glass tubes (14×100 mm.) and pipettes were used for each shake test. The sample was mixed before pipetting 1·0, 0·75, 0·5 and 0·25 ml of amniotic fluid into four tubes. The tubes containing 0·75, 0·5 and 0·25 ml. of amniotic fluid were made up to exactly 1 ml. by adding 0·9 per cent saline. One ml. of a solution of 95 per cent ethanol was added to each tube. The tubes were then held against a mechanical vibrator for 15 seconds and placed on a rack not subject to vibration. After exactly 15 minutes the tubes were viewed against a dark background with an overhead light and the presence of a continuous ring of bubbles around the meniscus was taken as a positive result for that tube. The final dilutions of the amniotic fluid in each tube were: Tube 1, 1 : 1; Tube 2, 1 : 1·3; Tube 3, 1 : 2, and Tube 4, 1 : 4. A *positive* shake test result was taken as a continuous ring of bubbles to a dilution of 1 : 2 or more (Clements *et al.*, 1972). An *intermediate* result meant the presence of a continuous ring of bubbles up to a dilution of 1 : 1·3, while a *negative* result means the absence of bubbles beyond a dilution of 1 : 1.

The palmitic acid levels in the amniotic fluid were measured by the method of Moore *et al.* (1975). Less than 25 μg./ml. is considered a low and "immature" level, between 25–35 μg./ml. an intermediate level, and above 35 μg./ml. a "mature" level. All babies born were observed for respiratory difficulties and the respiratory distress syndrome was diagnosed in the prseence of inspiratory retraction, expiratory grunting, poor alveolar entry, hypoxaemia as demonstrated by blood gas analysis and characteristic X-ray appearances.

## RESULTS

The variation of the results of the four observers is shown in Table I. Sixty-eight out of 106 results (64 per cent) correlated to within one tube or less; the remainder differed by more than one tube. The greatest variation in shake test results occurred in samples with palmitic acid levels between 25 and 50 μg./ml.

TABLE I

*Variation in results obtained by four observers*

| Variance | Number | Per cent |
|---|---|---|
| All 4 observers agreed | 36 | 34·0 |
| Results varied by 1 tube | 32 | 30·2 |
| Results varied by 2 tubes | 28 | 26·4 |
| Results varied by 3 tubes | 10 | 9·4 |
| Results varied by 4 tubes | 0 | 0 |

In the cases where all four observers agreed on the shake test result, the results were compared with the palmitic acid level (Table II).

TABLE II

*Correlation of palmitic acid levels with cases in which all four observers agreed on the shake test result*

| Palmitic acid level (μg./ml.) | Shake test negative with no bubbles or bubbles in first tube | Shake test positive with bubbles in first two tubes | Shake test positive with bubble in first three tubes or all four tubes |
|---|---|---|---|
| less than 25 | 31 | 0 | 0 |
| 25–35 | 4 | 0 | 0 |
| more than 35 | 4 | 17 | 12 |

TABLE III

*Individual shake test results correlated with the palmitic acid level*

| Palmitic acid level (μg./ml.) | Total number of samples in each group | Negative with no bubbles | Negative with bubbles in the first tube | Bubbles in the first two tubes | Positive with bubbles in the first three tubes | Positive with bubbles in all four tubes |
|---|---|---|---|---|---|---|
| Less than 25 per ml | 144 | 117 | 21 | 5 | 1 | 0 |
| 25 to 35 | 48 | 21 | 10 | 13 | 4 | 0 |
| More than 35 | 232 | 20 | 30 | 67 | 84 | 31 |

In 39 cases the observers all agreed that the shake test was *negative* and in 35 instances the palmitic acid level was 35 μg./ml. or less. In the 12 cases where the shake test case came out *positive* and in the 17 cases in which the result was intermediate the palmitic acid level was always more than 35 μg./ml. Thus, when the four observers agreed, no false positive results were obtained, whereas four out of 39 (10 per cent) of the samples in this group gave false negative results.

The 424 individual shake tests are correlated with the palmitic acid levels in Table III. When all four tubes showed bubbles (31 samples) the palmitic acid level was always over 35 μg./ml. When the first three tubes showed bubbles, five out of 84 of the samples had palmitic acid levels below 35 μg./ml., making a false positive rate of 5·6 per cent and when only the first two tubes showed bubbles, 18 out of 85 (21.2 per cent) of the samples had palmitic acid levels below 35 μg./ml.

Alternatively, when no tubes showed bubbles, 20 out of 158 of the samples had palmitic acid levels higher than 35 μg./ml. making a false negative rate of 12·7 per cent and when bubbles were seen in the first tube 30 out of 61 of the samples later proved to have palmitic acid levels over 35 μg./ml. making a false negative rate of 49·2 per cent. Thus, in relation to the shake test result, the percentage of samples that later proved to have mature palmitic acid levels were as follows: no bubbles, 12·7 per cent; bubbles in the first tube, 49·2 per cent; bubbles in the first two tubes, 78·8 per cent; bubbles in the first three tubes, 94·4 per cent, and bubbles in all four tubes, 100 per cent.

There were 48 amniotic fluid samples with palmitic acid levels of 35 μg./ml. or less and 13 of these patients delivered within 24 hours of the collection of the sample (Table IV). Two of the pregnancies were terminated prematurely because of anencephaly. Patient 2 was delivered

TABLE IV

*Patients with amniotic fluid palmitic acid levels under 35 μg./ml. who were delivered within 24 hours of amniocentesis or artificial rupture of membranes (ARM). (RDS = respiratory distress syndrome)*

| Patient No. | Source of amniotic fluid sample | Palmitic acid level (μg./ml.) | Outcome |
|---|---|---|---|
| 1 | ARM | less than 5 | Anencephalic |
| 2 | Amniocentesis | 6 | Severe RDS |
| 3 | ARM | 8 | Anencephalic |
| 4 | ARM | 9 | No RDS |
| 5 | ARM | 15 | Mild RDS |
| 6 | ARM | 21 | No RDS |
| 7 | ARM | 22 | No RDS |
| 8 | ARM | 22 | No RDS |
| 9 | ARM | 25 | No RDS |
| 10 | Amniocentesis | 25 | Mild RDS |
| 11 | ARM | 27 | No RDS |
| 12 | ARM | 28 | No RDS |
| 13 | ARM | 32 | No RDS |

by Caesarean section at 34 weeks gestation because of severe rhesus isoimmunization, patient 5 was induced at 39 weeks gestation and patient 10, a diabetic requiring high doses of insulin, laboured spontaneously at 36 weeks gestation. These three patients were the only ones in which the infants developed a respiratory distress syndrome.

Excluding the anencephalic fetuses, three out of eleven babies delivered with amniotic fluid palmitic acid levels below 35 $\mu$g./ml. developed the respiratory distress syndrome. The highest level that was associated with a mild respiratory distress syndrome was 25 $\mu$g./ml. and the lowest value in which the baby had no respiratory distress was 9 $\mu$g./ml. The shake tests performed on the amniotic fluid from patients 2, 5 and 10 were all negative.

## DISCUSSION

Variation in the results of the shake test performed by different observers on the same sample appears to be maximal in an intermediate range of palmitic acid values. When a high or low palmitic acid was present there was good agreement between the observers' results. This finding confirms the results of Boehm *et al.* (1973) who found that the shake test correlated best with clearly mature or immature lecithin/sphyngomyelin ratios. When a positive result is obtained on repeated shake tests on the same sample, a reliable correlation with mature levels of palmitic acid can be expected. Similarly, a single shake test giving a positive result with bubbles in all four tubes appears to be reliable. A fifth tube and dilution as described originally (Clements *et al.*, 1972) therefore seems unnecessary.

When a positive shake test was obtained with bubbles in only the first three tubes, we found a 5·6 per cent false positive rate as compared with the palmitic acid level. However, Wagstaff and Bromham (1973) compared the shake test to the lecithin/sphyngomyelin ratio and found no ratio below 2·0 in 96 cases with a positive shake test at 1 : 2 dilution, although one of the infants born did develop "hyaline membrane disease". In the same series a false negative rate of 1 out of 52 (1·9 per cent) was reported where the shake test produced bubbles, in one tube or less. Judged by comparison with palmitic acid levels our series showed a 12·7 per cent rate of false negatives when all four tubes were without bubbles and a 49·2 per cent incidence of false negatives when the first tube showed bubbles.

In this series, only half the samples with a palmitic acid level over 35 $\mu$g./ml. gave a positive shake test. If one changed the requirement for a positive shake test to the presence of bubbles in the first two tubes one would detect approximately three-quarters of the mature palmitic acid levels but this would introduce a 21 per cent false positive rate. Where the shake test gives an intermediate result and more sophisticated tests are unavailable it may be helpful to know the percentage likelihood of a mature level or surfactant and this in some cases may be helpfully related to the clinical risk of a continuing pregnancy.

Respiratory distress syndrome occurred in the infants of three of the eleven patients with low amniotic fluid palmitic acid levels. Further studies are necessary to predict the risk of respiratory distress syndrome in relation to low palmitic acid levels.

Despite an element of observer variation the shake test, when performed in the clinical side room by the method described on samples uncontaminated by blood or meconium, gives reliable results when positive but is unreliable when negative. When results in the intermediate range are obtained an estimate of the clinical risk of an immature level of surfactant can be given. However, a low surfactant level does not always predict respiratory distress syndrome.

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# THE ORIGIN OF AMNIOTIC FLUID LECITHIN

BY

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## Summary

Lecithin has been measured in amniotic fluid, pharyngeal aspirate, fetal and maternal plasma and fetal membranes from the same pregnancy. In the amniotic fluid from a term pregnancy 79 per cent of the lecithin is found in the reconstituted precipitate of centrifuged fluid. It is suggested that the lecithin of amniotic fluid may originate from sources other than fetal lung and that lecithin concentration is therefore a measure of overall fetal maturity.

FOLLOWING publications by Nelson (1969), Gluck *et al.* (1971) and Bhagwanani *et al.* (1972*a* and *b*), many centres are using lecithin-sphingomyelin ratios or lecithin concentrations in amniotic fluid to determine the likelihood of the newborn infant developing respiratory distress syndrome. These authors, together with Biggs *et al.* (1974), suggested that amniotic fluid phospholipids originate principally in the fetal lung and that their presence in sufficient quantities in amniotic fluid shows that the fetal lung has attained maturity.

Roux *et al.* (1973 and 1974) pointed out that lecithin and sphingomyelin could be found both in the pharyngeal aspirate and amnio-chorion. This paper confirms these findings and shows that lecithin is also present in fetal and maternal plasma, thus suggesting that some of the lecithin found in amniotic fluid may not originate from fetal lungs.

## METHODS

Amniotic fluid from normal pregnancies was collected at artificial rupture of the membranes using a Drewe-Smythe catheter. Care was taken to avoid contamination with obstetric cream, and only fluids free from blood and meconium were collected (Wagstaff *et al.*, 1974). A measured volume of the fluid was centrifuged at 3000 *g* and 4 °C. for ten minutes. The clear supernatant was removed and the deposit resuspended in the original fluid volume using distilled water.

At eight term deliveries, maternal blood and fetal blood (from the placenta) were collected in heparinized containers, centrifuged and the plasma separated. Infant pharyngeal aspirate was collected and, where possible, fetal urine. Aliquots of all specimens were immediately placed in 3 ml. of methanol-chloroform (2 : 1). The volumes of fluid used were: amniotic fluid (uncentrifuged, centrifuged and resuspended deposit), 0·8 ml.; pharyngeal aspirate 0·1 ml.; fetal plasma, 0·3 ml.; maternal plasma, 0·1 ml.; and fetal urine 1 ml. Whenever possible, samples were duplicated. The portions of amnio-chorion used were about 1 cm. square. Total lecithin concentration was measured by the method of Bhagwanani *et al.* (1972*a* and *b*). Two minor

TABLE I

*Lecithin concentration in fluids and tissues examined*

| Patient | Lecithin concentration (mg./100 ml.) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Amniotic fluid | | Fetal membrane | Plasma | | Pharyngeal aspirate | Fetal urine |
| | Uncentrifuged | Centrifuged | | Fetal | Maternal | | |
| Y.C. | 11·9 | 3·0 | 382·4 | 49·3 | 174·0 | 28·0 | 1·0 |
| | 11·6 | 3·3 | 264·2 | 48·3 | 183·4 | 23·0 | 0·4 |
| O.F. | 9·9 | 1·8 | 273·4 | 55·6 | 153·0 | – | 0·7 |
| | 8·6 | 1·6 | 219·5 | – | 231·0 | – | 0·7 |
| M.N. | 17·0 | 2·4 | 359·1 | 23·5 | 166·0 | 165·6 | 0·6 |
| | 14·8 | 1·6 | 543·3 | 16·8 | 182·0 | 165·6 | 0·5 |
| M.G. | 20·3 | 0·8 | 269·7 | 60·9 | 190·4 | – | – |
| | 17·9 | 1·1 | – | 61·3 | 173·2 | – | – |
| B.M. | 11·2 | 1·1 | 353·5 | 71·7 | 153·0 | 68·4 | – |
| | 6·8 | 0·9 | 364·4 | 70·0 | 158·6 | 58·2 | – |
| B.C. | 14·8 | 1·4 | 119·1 | 45·9 | 188·8 | – | – |
| | 13·6 | 1·3 | 127·5 | 52·5 | 203·2 | – | – |
| R.S. | 9·7 | 3·2 | 264·4 | 59·2 | 192·4 | 60·0 | – |
| | 7·0 | 2·9 | 253·7 | 58·9 | 163·2 | 46·8 | – |
| R.F. | 16·8 | 3·5 | 454·9 | 48·0 | 153·0 | 113·4 | – |
| | 14·2 | 3·1 | 445·0 | 41·7 | 150·0 | 104·0 | – |

modifications were that the chloroform extract was blown to dryness at 80 °C. and solvent system I contained chloroform/methanol/ammonia/water in proportions of 60/30/4/0·5.

An aliquot of each lecithin eluate used for phosphorus estimation was also taken for palmitic acid measurement by gas liquid chromatography (GLC). Heptadecanoic acid was added as internal standard. After alkaline hydrolysis and extraction into hexane the free fatty acids were treated with diazomethane. The methyl esters were taken up in hexane and separated isothermally at 180 °C. on a six-foot column containing 15 per cent diethylene glycol succinate on Chromosorb W in a Hewlett-Packard 402 gas chromatograph with flame ionization detector. Pure methyl esters of long chain fatty acids from Sigma (London) were used as standards for quantitation.

## RESULTS

The lecithin concentrations found in amniotic fluid, fetal membranes, fetal and maternal plasma, newborn pharyngeal aspirate and fetal urine are shown in Table I. Large amounts of lecithin were found in fetal membranes and in both fetal and maternal plasma. Pharyngeal aspirate contained varying amounts of lecithin but it is uncertain whether the amounts found in fetal urine were of significance. There was a considerable drop in the lecithin concentration of amniotic fluid when centrifuged. Table II

TABLE II

*Lecithin concentration in amniotic fluid*

| Patient | Lecithin concentration in amniotic fluid (mg./100 ml.) | | |
|---|---|---|---|
| | Uncentrifuged | Centrifuged | Reconstituted precipitate |
| G.S. | 9·1 | 2·8 | 8·5 |
| | 7·7 | 2·5 | 8·1 |
| I.C. | 3·6 | 0·9 | 4·3 |
| | 2·4 | 0·6 | 3·2 |
| E.D. | 7·6 | 1·5 | 7·5 |
| | 7·2 | 1·1 | 7·3 |
| A.M. | 19·3 | 3·6 | 17·5 |
| | 19·2 | 3·5 | 16·2 |
| F.W. | 7·6 | 2·9 | 7·2 |
| | 7·4 | 2·4 | 7·2 |
| H.W. | 5·3 | 2·3 | 6·7 |
| | 5·3 | 1·6 | 6·0 |
| R.O. | 13·4 | 4·1 | 11·1 |
| | 12·8 | 3·4 | 9·7 |
| R.S. | 9·7 | 3·2 | 6·4 |
| | 7·0 | 2·9 | 5·9 |
| R.F. | 16·8 | 3·5 | 11·4 |
| | 14·2 | 3·1 | 10·8 |

TABLE III

*Palmitic acid concentration in fluids and tissues examined*

| Patient | Palmitic acid concentration (mmol./L.) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Amniotic fluid | | Fetal membrane | Plasma | | Pharyngeal aspirate | Fetal urine |
| | Uncentrifuged | Centrifuged | | Fetal | Maternal | | |
| Y.C. | 0·193 | 0·033 | 2·519 | 0·516 | 1·707 | 0·593 | 0·053 |
| | 0·173 | 0·040 | 2·239 | 0·445 | 1·973 | 0·393 | 0·021 |
| O.F. | 0·167 | 0·027 | 2·664 | 0·427 | 1·387 | – | 0·021 |
| | 0·143 | 0·020 | 2·442 | – | 2·027 | – | 0·011 |
| M.N. | 0·307 | 0·053 | 2·640 | 0·142 | 1·440 | 2·933 | 0·016 |
| | 0·287 | 0·047 | 2·218 | 0·124 | 1·600 | 2·667 | 0·023 |
| M.G. | 0·367 | 0·053 | 0·875 | 0·427 | 1·493 | – | – |
| | 0·305 | 0·040 | – | 0·427 | 1·280 | – | – |
| B.M. | 0·180 | 0·037 | 2·187 | 0·480 | 1·120 | 0·907 | – |
| | 0·120 | 0·013 | 2·346 | 0·462 | 1·280 | 0·853 | – |
| B.C. | 0·354 | 0·057 | 0·919 | 0·338 | 1·440 | – | – |
| | 0·320 | 0·047 | 0·984 | 0·391 | 1·440 | – | – |
| R.S. | 0·107 | 0·040 | 1·687 | 0·391 | 1·493 | 0·533 | – |
| | 0·100 | 0·027 | 1·675 | 0·348 | 1·227 | 0·480 | – |
| R.F. | 0·233 | 0·040 | 3·225 | 0·320 | 1·228 | 1·607 | – |
| | 0·220 | 0·033 | 2·779 | 0·320 | 1·013 | 1·333 | – |

TABLE IV

*Palmitic acid concentration in amniotic fluid*

| Patient | Palmitic acid concentration in amniotic fluid (mmol./litre) | | |
|---|---|---|---|
| | Uncentrifuged fluid | Centrifuged fluid supernatant | Resuspended deposit |
| G.S. | 0·140 | 0·043 | 0·153 |
| | 0·127 | 0·027 | 0·133 |
| I.C. | 0·063 | 0·033 | 0·053 |
| | 0·057 | 0·029 | 0·047 |
| E.D. | 0·127 | 0·043 | 0·113 |
| | 0·129 | 0·033 | 0·101 |
| A.M. | 0·360 | 0·080 | 0·267 |
| | 0·324 | 0·073 | 0·300 |
| F.W. | 0·133 | 0·037 | 0·123 |
| | 0·147 | 0·047 | 0·143 |
| H.W. | 0·093 | 0·026 | 0·101 |
| | 0·087 | 0·035 | 0·107 |
| R.O. | 0·193 | 0·039 | 0·122 |
| | 0·174 | 0·036 | 0·132 |
| R.S. | 0·107 | 0·040 | 0·080 |
| | 0·100 | 0·027 | 0·067 |
| R.F. | 0·233 | 0·040 | 0·147 |
| | 0·220 | 0·033 | 0·170 |

shows the concentration of lecithin in samples of amniotic fluid when the fluid was examined in the uncentrifuged state, following centrifugation and when the precipitate was reconstituted with distilled water. The reconstituted precipitate contained most of the lecithin found in the uncentrifuged fluid.

Tables III and IV show the palmitic acid concentrations found in the lecithin fractions of the same samples as shown in Tables I and II. If all of the palmitic acid found were assumed to arise from dipalmitoyl lecithin then for amniotic fluid the mean dipalmitoyl lecithin concentration would be almost 70 per cent of the value calculated from phosphorus measurements, for pharyngeal aspirate it would be about 50 per cent, and for fetal or maternal plasma and fetal membranes about 30 per cent.

## DISCUSSION

These results show that lecithin as measured by the method of Bhagwanani *et al.* (1972*a* and *b*) is found in amniotic fluid, maternal and fetal plasma and that it can be extracted from fetal membranes. The results confirm those of Gluck *et al.* (1971) and Biggs *et al.* (1974) that lecithin is found in pharyngeal aspirate but this does not in itself prove that the lecithin found in amniotic fluid originates solely in fetal lung. Lecithin is found in specimens of amniotic fluid both uncentrifuged and centrifuged but it is interesting to note that between 71 to 87 per cent of the total lecithin is found in the reconstituted precipitate. Some preliminary experiments were performed in which L/S ratios were estimated before and after centrifugation. The ratios were considerably higher in the amniotic fluid before centrifugation. It is likely that the deposit obtained in centrifugation, which contains the cellular content of the amniotic fluid, originates from membrane, cord and the fetus (skin, mouth, bladder, and vagina).

Palmitic acid was found in every fraction examined, but only in the case of amniotic fluid did the dipalmitoyl lecithin concentration calculated from the palmitic acid content agree reasonably well with the concentration calculated from the phosphorus content. This would imply that with complex materials, such as plasma, aspirate and membrane, the eluate from the lecithin area of the thin layer chromatography plate includes phosphorus containing substances other than dipalmitoyl lecithin. Inspection of the GLC charts suggests also that although palmitic acid is the predominant long chain fatty acid in extracts from plasma, aspirate and membranes, as in amniotic fluid extracts, the former contain higher concentrations of other acids (myristic, stearic, oleic) than amniotic fluid extracts.

Nevertheless, lecithin in amniotic fluid could contain material from sources other than fetal lung and it may be that the lecithin concentration of term amniotic fluid is a biochemical measure of the cellular content of the fluid.

These results confirm the belief of Roux *et al.* (1973) and Condorelli *et al.* (1974) that the lecithin found in amniotic fluid is of multiple origin. These findings do not destroy the value of lecithin concentration in amniotic fluid as a clinical test but suggest that care should be taken in its interpretation.

## ACKNOWLEDGEMENTS

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# FETAL HEART RATE MONITORING DURING BREECH LABOUR

BY

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## Summary

Forty-two breech deliveries were monitored throughout labour. Serious asphyxia at birth occurred in 15 babies, in every case preceded by abnormalities in the fetal heart rate. Interpretation of the records implicated umbilical cord compression as the likely cause of asphyxia.

FETAL heart rate (FHR) monitoring is now established as an important technique in the early detection of fetal asphyxia, but published work has largely related to the cephalic presentation.

Breech deliveries are of particular interest since fetal asphyxia may develop rapidly and be an important factor in determining the outcome. Three distinctions may at once be made in contrast to the cephalic presentation. Firstly, patients with breech presentation selected for vaginal delivery do not usually have other high risk factors. Secondly, the incidence of umbilical cord prolapse is higher, and thirdly, head compression, which is known to cause important changes in the FHR, does not occur until the end of the second stage.

## METHODS

Forty-two patients with breech presentations, delivered between 1971 and 1973 at King's College Hospital, were reviewed. The patients were selected if the FHR had been monitored and vaginal delivery achieved. There were 32 primigravidae and 10 multiparae. Seven infants were first twins, the remainder were singletons. Thirteen patients had mild pre-eclampsia (diastolic blood pressure less than 100 mm. Hg, mild oedema, no proteinuria), one patient was an elderly primigravida aged 35 and three patients were delivered prematurely at 31, 33 and 36 weeks respectively. Eight infants weighed between 1000 and 2449 g. and 34 between 2500 and 4000 g. There were four cases of cord prolapse.

A clip or spiral electrode was attached to the buttock as soon as practicable and thereafter the FHR was recorded on paper at 1 cm. per minute with a Hewlett Packard 8020A fetal heart monitor. Contractions were recorded by an external tocometer placed on the maternal abdomen. During the second stage of labour the descent of the breech sometimes disturbed the contact of the electrode with the mother, producing some loss of data. This could be reduced by making an alternative maternal contact on the thigh. The average duration of each labour was 9·8 hours and the average time of FHR recording during labour was 6·2 hours.

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All Apgar scores were measured at one minute.

All FHR records obtained during the first stage of labour were analysed (Table I). In the records described as normal the heart rate lay between 120 and 160 beats per minute between and during contractions. Heart rates of less than 120 or more than 160 between contractions were designated baseline bradycardia and baseline tachycardia respectively. All the decelerations recorded were of the variable type (Hon and Quilligan, 1967). Loss of beat-to-beat variation was defined as a variation of baseline heart rate of less than 5 beats in any minute. Excessive beat-to-beat variation was defined as a variation in the baseline heart rate greater than 25 beats in any minute. These changes were equivalent to the silent and saltatory patterns described by Hammacher *et al.* (1968).

## RESULTS

The patients were divided into two groups: those in which the fetus was in satisfactory condition at birth and those in which the fetus was born seriously depressed (Apgar score of 5 or less at one minute, or intubation required, or both); 15 patients fell into the latter group.

Table I shows the overall results. The fifteen babies born in poor condition all showed abnormal FHR patterns during the first stage

TABLE I

*Fetal heart rate patterns during the first stage of labour related to the Apgar score at one minute.* ● = *satisfactory birth condition.* ○ = *depressed birth condition.* ⧇ = *excessive beat-to-beat variation (saltatory pattern)*

| | APGAR SCORE | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| **normal** | | | | | | ●<br>●<br>● | ●<br>●<br>●<br>●<br>● | ●<br>●<br>●<br>●<br>● | ●<br>●<br>●<br>● | |
| **baseline bradycardia** | | | | | | | ● | ● | ● | |
| **baseline tachycardia** | | | | | ○<br>⧇ | | ● | | | |
| **normal baseline + decelerations** | | | | | | | | ● | ● | |
| **baseline bradycardia + decelerations** | | | | | ○<br>○ | | | | | |
| **baseline tachycardia + decelerations** | | ○ | | | ○<br>○ | | ● | ● | | |
| **loss of beat-to-beat variation + baseline tachycardia** | | ○ | | | | ○ | | ● | | |
| **loss of beat-to-beat variation + decelerations** | | | ○ | | | | | | | |
| **loss of beat-to-beat variation + baseline tachycardia + decelerations** | ○<br>⧇ | ○ | | ○ | ○ | ● | | | | |

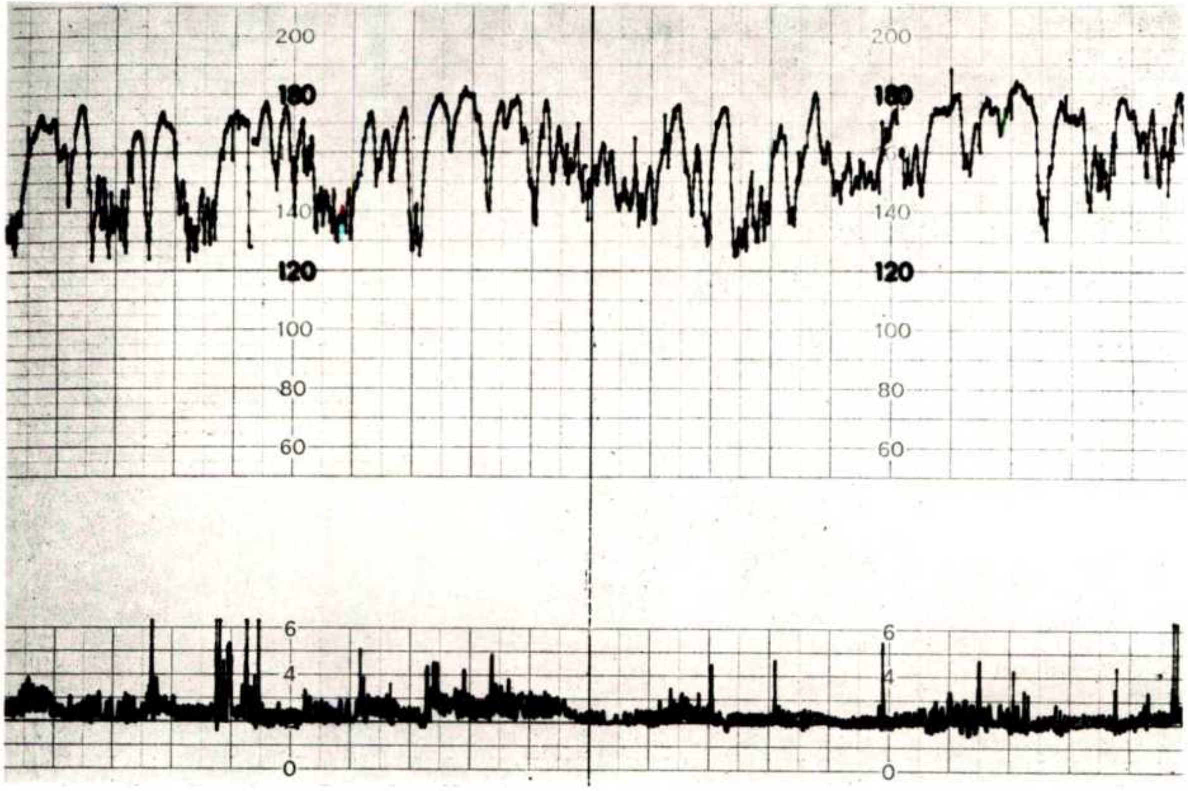

FIG. 1

FHR showing excessive beat-to-beat variation (saltatory pattern). Assisted breech delivery 4½ hours later. Apgar score 5. Weight 2800 g. (39 weeks gestation). Record duration 20 minutes, recording speed 1 cm. per minute (as in Figs. 2, 3 and 5).

Almost all (12 out of 15) showed a tachycardia, eleven showed variable decelerations and eight showed loss of beat-to-beat variation. In most cases (13 out of 15) two or three abnormalities were present. The abnormalities were present for periods of time ranging from 15 minutes to 10½ hours. Most abnormalities of the FHR (9 out of 15) lasted for less than 2 hours. In addition, two patients showed episodes of excessive beat-to-beat variation lasting up to one hour (Figure 1). All four patients with cord prolapse showed a baseline tachycardia, three showed loss of beat-to-beat variation and three showed variable decelerations. An example of the FHR pattern in one of these patients is shown in Figure 2.

Seventeen patients (40 per cent) showed a short lasting tachycardia (acceleration) during the contraction periods at some point in the first stage of labour. This was not significantly related to fetal asphyxia. An extract from a patient with a remarkable series of accelerations over a period of eight hours is shown in Figure 3. Ten patients (see Table I) showed abnormal FHR patterns but were born in satisfactory condition. The abnormalities were present from one to three hours before the onset of the second stage.

It was possible to examine the FHR records during the second stage of labour in 30 patients, 15 records being from the group of patients whose baby was in poor condition at birth. Seventy-five per cent of these records showed a tachycardia with variable decelerations no matter which group they came from. Loss of beat-to-beat variation was found in nine patients, including six whose baby was born in poor condition. As in the study of Teteris *et al.*

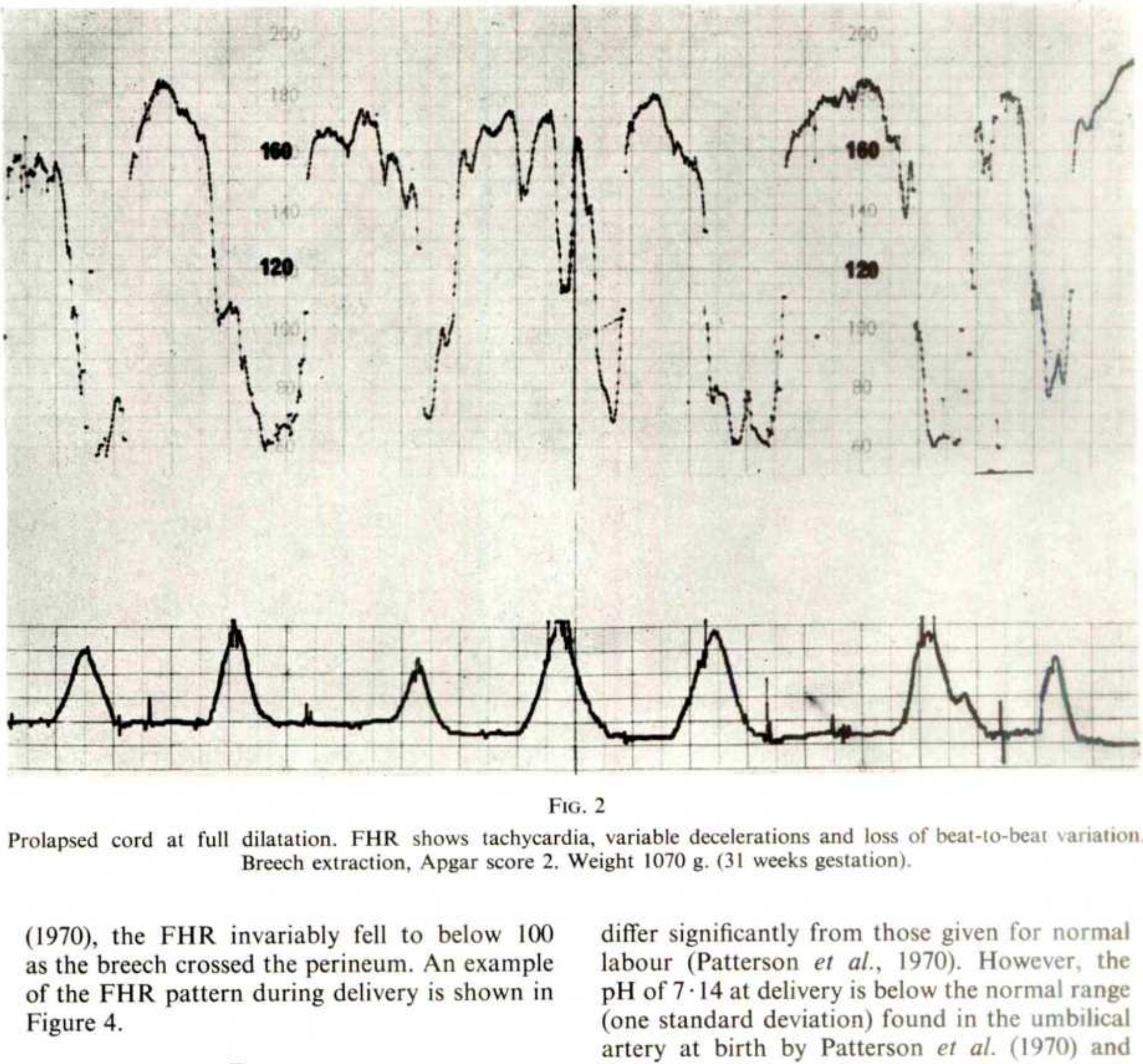

FIG. 2

Prolapsed cord at full dilatation. FHR shows tachycardia, variable decelerations and loss of beat-to-beat variation. Breech extraction, Apgar score 2. Weight 1070 g. (31 weeks gestation).

(1970), the FHR invariably fell to below 100 as the breech crossed the perineum. An example of the FHR pattern during delivery is shown in Figure 4.

## DISCUSSION

Relatively little study has been made of the development of fetal asphyxia during breech labour. The status of fetal pH measurement was investigated by Eliot and Hill (1972). Averaged pH values, obtained from 25 patients in that study, showed that there was a small decrease between the first stage value (pH 7·29) and the arrival of the breech on the perineum (pH 7·25) but thereafter there was a greater fall up to the point of delivery (pH 7·14). Although no previous study has been made to establish the normal range of pH for the breech presentation, pH values at 7·29 and 7·24 do not appear to differ significantly from those given for normal labour (Patterson *et al.*, 1970). However, the pH of 7·14 at delivery is below the normal range (one standard deviation) found in the umbilical artery at birth by Patterson *et al.* (1970) and probably indicates the greater degree of asphyxia that is believed to accompany breech deliveries. Even so, the pH of blood obtained from the buttocks or feet may not be representative of that perfusing the vital centres (Saling, 1970), particularly during the second stage of labour.

Eliot and Hill (1972) suggested that the fall in pH during the last part of a breech delivery was caused by cord compression and this seems to be the most likely explanation for the abnormal FHR patterns in this series. Firstly, although early decelerations (Hon and Quilligan, 1967) were occasionally recorded as isolated events, the typical deceleration was variable and these

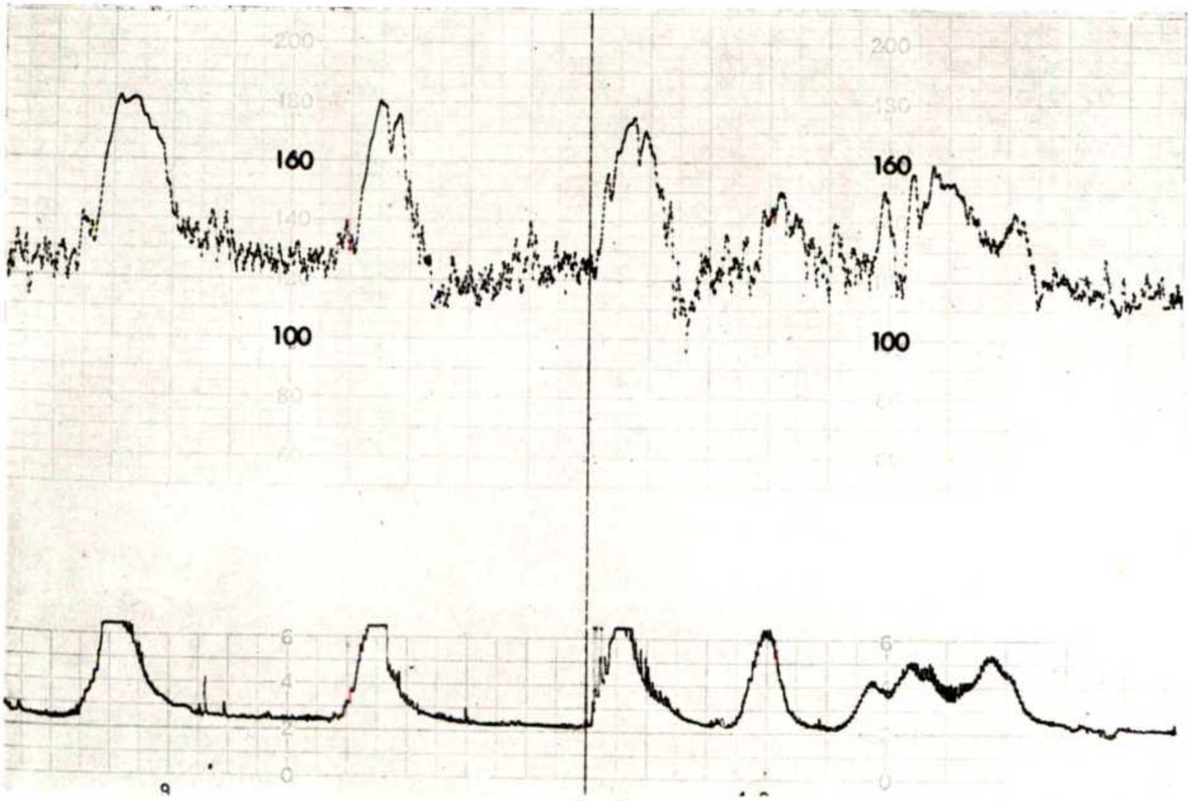

FIG. 3
FHR showing accelerations. Breech extraction 5 hours later for poor progress. Apgar score 6. Weight 2550 g. (36½ weeks gestation).

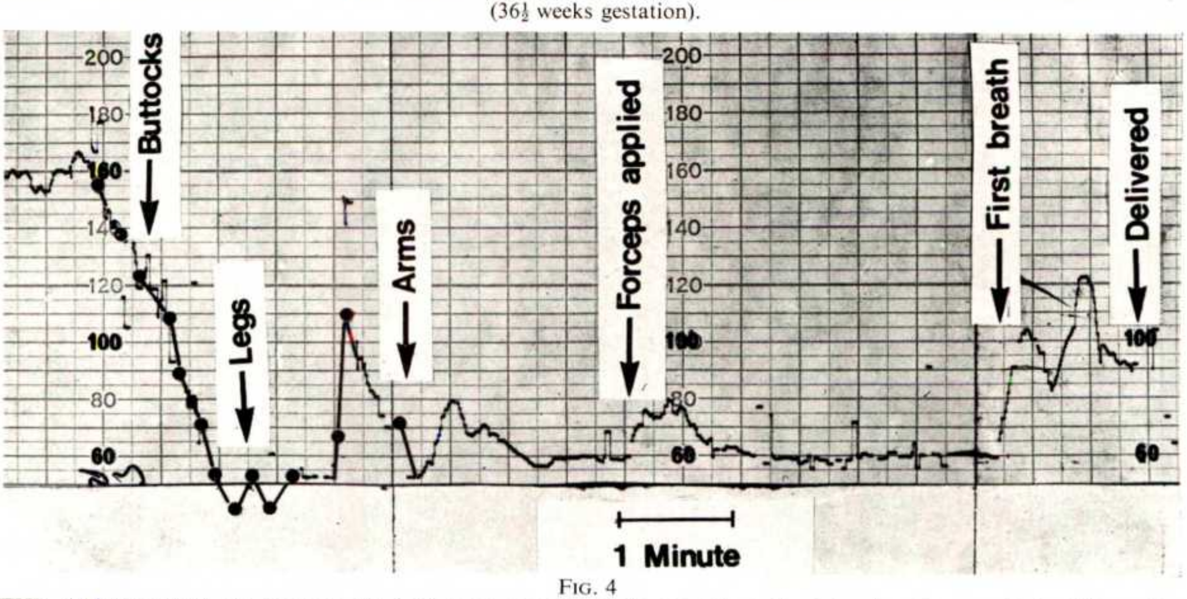

FIG. 4
FHR during delivery. In addition the fetal ECG was recorded separately and points plotted ● are obtained from this. Assisted breech delivery, Apgar score 9. Weight 3090 g. (39 weeks gestation). Note: recording speed 2 cm. per minute.

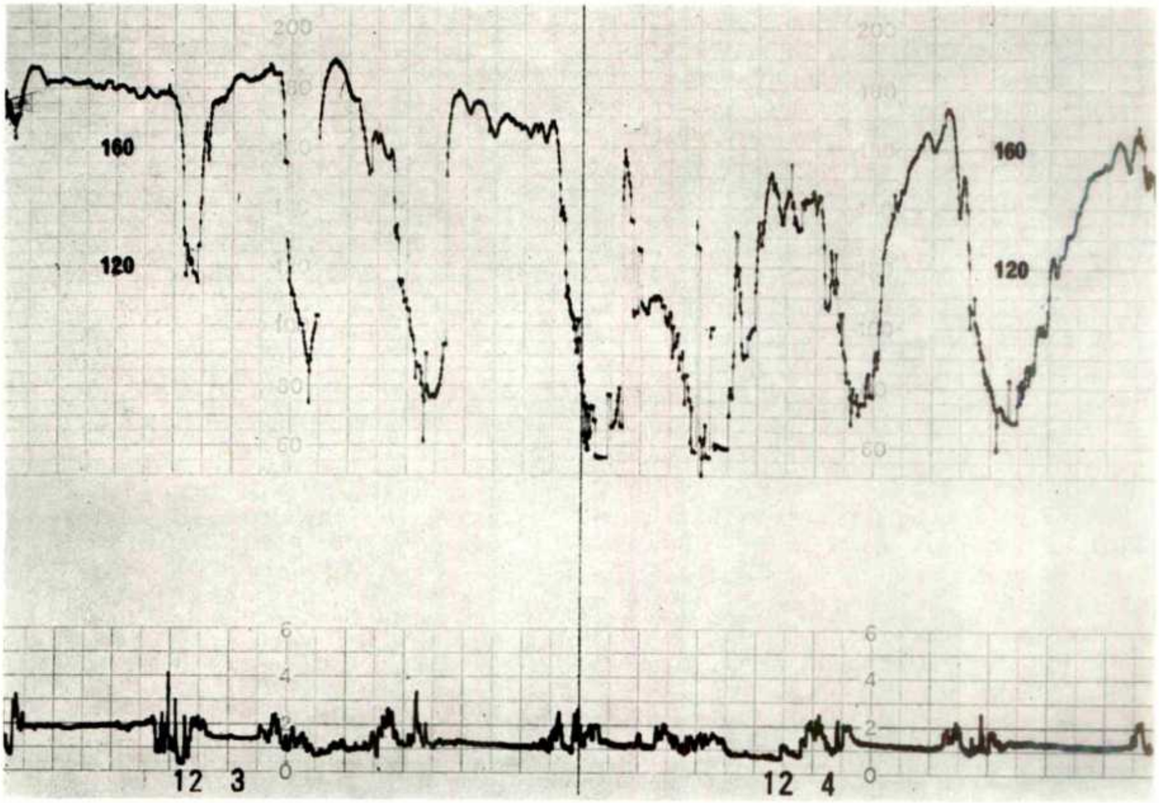

FIG. 5
FHR at full dilatation showing tachycardia, variable decelerations and loss of beat-to-beat variation. Assisted breech delivery, Apgar score 1. Weight 2970 g. (40 weeks gestation).

have been produced experimentally by cord compression (Lee and Hon, 1963). Secondly, there was a striking similarity between the records associated with cord prolapse, when cord compression was likely to exist, and those records from the other cases of asphyxia (Figure 5). Finally, episodes of excessive beat-to-beat variation, which were observed in this series, have been attributed to cord complications by Hammacher *et al.* (1968). The FHR records also showed that asphyxia developed before the arrival of the breech on the perineum; in fact the usual time for this to start was during the accelerated phase of labour as the breech descended through the pelvis.

Little appears to be known of the cause or significance of the acceleration pattern in the fetal heart rate. The study of Beard *et al.* (1971), relating the fetal pH to the FHR pattern, included 20 records of accelerations and all were associated with a normal pH. An analysis was made of 100 randomly selected FHR records from patients with a fetus in cephalic presentation; this showed the incidence of acceleration patterns to be only 21 per cent compared to 40 per cent in this series of breech labours. One could speculate that they could in some way be due to abdominal compression of the fetus during contractions.

During the first stage, abnormalities in the FHR indicate the need to examine the patient to exclude cord prolapse. Because such abnormalities may last for some hours and are not always related to serious depression of the newborn, measurement of the fetal pH at this juncture may be helpful. During the second stage, tachycardia and variable decelerations are to be expected and their appearance before this is important. Loss of beat-to-beat variation is a serious sign at any point. In this series the pH was only measured five times at full dilatation. In three cases the fetus was born in poor condition when the pH values had been 7·31, 7·30 and 7·10 respectively. In two cases the fetus was born in satisfactory condition after pH values of 7·27 and 7·07 respectively. Clearly, for the fetal pH to be predictive it should be obtained as close to delivery as possible. Eliot and Hill (1972) have suggested that the pH be done "at five-minute intervals from the time the breech reaches the perineum until the legs are disengaged". The staff required to implement such a scheme effectively make it unlikely to be adopted and their supposition that the fetal pH will fall before any change in the FHR would seem to be most unlikely from the evidence in this paper.

The ideal management may be a combination of continuous FHR monitoring and fetal pH measurement, but the outcome of a breech delivery also depends upon the delivery of the aftercoming head and as Eliot and Hill (1972) have pointed out, the chance of cerebral damage as the head is delivered may be increased by the presence of asphyxia. The dilemma of how to deliver the asphyxiated breech at full dilatation still remains. One of the babies in our series was delivered at term and has developed cerebral athetosis. The FHR had been normal until 45 minutes before delivery when a baseline tachycardia with loss of beat-to-beat variation developed. Large variable decelerations appeared 10 minutes later (Fig. 5). Full dilatation was diagnosed and an assisted breech delivery completed within 15 minutes. The umbilical cord was found around the trunk and neck and the birth weight was 2970 g. It is possible that such patients are best delivered by emergency Caesarean section.

## ACKNOWLEDGEMENT

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11TH AUSTRALIAN CONGRESS IN OBSTETRICS AND GYNAECOLOGY

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# BIOCHEMICAL AND HAEMATOLOGICAL CHANGES DURING THE INDUCTION OF LABOUR AT TERM WITH OXYTOCIN, PROSTAGLANDIN $E_2$ AND PROSTAGLANDIN $F_{2\alpha}$

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## Summary

A total of 75 patients had labour induced near term using intravenous oxytocin, prostaglandin $E_2$ or prostaglandin $F_{2\alpha}$. Biochemical and haematological investigations were performed to assess hepatic, renal and adrenocortical function and changes in platelet adhesiveness. In doses which were similarly effective in inducing labour, both prostaglandins were without the water-retaining effect of oxytocin. Adrenocortical stimulation was greatest with oxytocin. No difference between these agents was observed for the other measurements made.

VARIOUS authors have used prostaglandins for the induction of labour in patients near term (Karim *et al.*, 1969 and 1970; Beazley *et al.*, 1970; Embrey, 1970). Their relative effectiveness is established (Anderson *et al.*, 1971; Beazley and Gillespie, 1971; Vakhariya and Sherman, 1972; Naismith *et al.*, 1973). This paper considers biochemical and haematological changes resulting from the use of oxytocin, prostaglandin $E_2$ ($PGE_2$) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in doses with similar effectiveness for the induction of labour. The investigation covers various aspects of hepatic, renal, and adrenocortical function.

## METHODS

The study was carried out in St. Mary's Hospital, Manchester. To be included in the trial patients had to have a gestational age of not less than 35 weeks, no previous Caesarean section or hysterotomy, no diabetes, cardiac or renal disease and no evidence of cephalopelvic disproportion. Twenty-five patients were assigned at random to each of three groups for induction with oxytocin, $PGE_2$ or $PGF_{2\alpha}$. Their informed consent and co-operation were obtained. The main indications for induction (Table I) were pre-eclampsia of mild to moderate severity, postmaturity (a gestational age on clinical and radiological assessment of more than 42 weeks), an apparently small-for-dates fetus, the presence of antibodies in a rhesus negative mother and placental abruption occurring after 37 weeks gestation.

On the day of induction the patient had breakfast consisting of one cup of tea and one slice of toast, following which she was instructed to empty her bladder fully. The specimen was retained for analysis and a record was made of the time of previous micturition. The patient was then sent from the antenatal ward to the delivery unit where a careful record of fluid balance was kept, only tea and water being allowed as oral intake. Induction was commenced at 0900 hours in every case, and prior to infusion of the test substance, blood (the "pre-induction" specimen in the Tables) was taken for a full blood count and platelet adhesive-

TABLE I

*Summary of patients studied*

| | Oxytocin | $PGE_2$ | $PGF_{2\alpha}$ |
|---|---|---|---|
| No. of patients | 24 | 25 | 25 |
| Mean parity | 1·36 | 1 24 | 0·80 |
| Mean gestational age (days) | 279 | 277 | 283 |
| Indication for induction: | | | |
| Pre-eclampsia | 10 | 9 | 10 |
| Postmaturity | 9 | 10 | 14 |
| Dysmaturity | 1 | 2 | 0 |
| Rhesus antibodies | 3 | 1 | 0 |
| Accidental haemorrhage | 0 | 0 | 1 |
| Social reasons | 1 | 3 | 0 |
| Mean interval between starting infusion and onset of labour (hours·minutes) | 2·00 | 3·00 | 2·15 |
| Mean interval ±SD between starting infusion and end of third stage (hours·minutes) | 8·15±5·32 | 8·05±4·50 | 8·55±5·46 |
| Number with ketonuria before labour | 2 | 3 | 1 |
| Number with ketonuria during labour | 7 | 12 | 11 |
| Mean interval ±SD between delivery and post-delivery sample (hours·minutes) | 16·35±3·58 | 17·50±3·58 | 16·45±5·26 |

ness studies as well as for measurements of serum electrolytes, creatinine, bilirubin, aspartate and alanine aminotransferases (GOT and GPT), alkaline phosphatase and plasma cortisol levels. The oxytocic agent was then administered by intravenous infusion. For oxytocin, a concentration of 10 u per 500 ml. of 5 per cent dextrose was used commencing with 1 mu/minute and doubling dosage every half hour until labour was established. For $PGF_{2\alpha}$ and $PGE_2$, 7·5 mg. and 0·75 mg. were respectively dissolved in 500 ml. of 5 per cent dextrose. Starting dosages for $PGF_{2\alpha}$ and $PGE_2$ were respectively 2·5 μg./minute and 0·25 μg./minute and, if necessary, dosages were doubled after 30 minutes and after 90 minutes. Once infusion commenced a No. 14 Foley catheter was inserted into the bladder aseptically and continuous drainage allowed, the urine collecting bags being changed four-hourly. If conditions were favourable, the membranes were ruptured at the time of bladder catheterization.

At 1200 hours, a second specimen of blood (the "during infusion" specimen) was taken for the tests enumerated earlier. Urine collection and fluid balance measurements were completed on delivery of the placenta. A 100 ml. of 1 per cent noxytiolin was then instilled into the bladder and the catheter spigotted and removed.

The volume, pH, sodium and potassium concentrations and osmolality of all urine specimens were measured and each specimen was tested qualitatively for the presence of blood, sugar, ketones and proteins.

After delivery a further 0900 hour specimen of blood ("post-delivery" specimen) was collected. Where delivery occurred after 0900 hours but before midnight the blood was collected on the following morning. If delivery occurred between midnight and 0900 hours, collection was postponed until the following day.

*Analytical methods*

Sodium, potassium, chloride and creatinine were measured in serum or urine using standard AutoAnalyzer methods. Alkaline phosphatase was determined on the AutoAnalyzer using phenyl phosphate as substrate. Serum bilirubin was determined using the method of Powell (1944), plasma cortisol using the method of Mattingly (1962). GOT and GPT were determined colorimetrically using kits (Diamed

Diagnostics, Liverpool) based on the original method of Reitman and Frankel (1957). Osmolalities were determined by freezing point depression using an Advanced Instruments Osmometer.

Platelet sensitivity to ADP was estimated by measuring the standard platelet aggregate filtration pressure (Sanderson *et al.*, 1973). Full blood counts were made with the Coulter S counter.

## RESULTS

### *Clinical findings*

A summary of the patients studied is shown in Table I; one patient was omitted from the original oxytocin group as labour ended in Caesarean section for fetal distress.

### *Haematological findings*

Standard platelet aggregate filtration pressure was measured in "pre-induction" and "during infusion" specimens. The differences examined by the "t" test, were not statistically significant (Table II). For $PGF_{2\alpha}$ because of small numbers the results were also tested by the Walsh test ($P>0 \cdot 094$). There was no significant difference in the pattern of response of red cell count, haemoglobin or white cell count when oxytocin was compared with either prostaglandin. All three oxytocic agents were associated with a similar rise in white cell count during labour.

TABLE II

*Change in standard platelet aggregate filtration pressure (SPAFP)*

| | "Pre-induction" SPAFP level minus "during infusion" SPAFP level (units) | | |
|---|---|---|---|
| | Oxytocin | $PGE_2$ | $PGF_{2\alpha}$ |
| Mean | +5 | −4 | −20 |
| SD | 93 | 60 | 144 |
| N | 20 | 21 | 13 |

None of the changes observed was significant.

### *Biochemical changes*

Usually the comparison was made between oxytocin and $PGF_{2\alpha}$ or oxytocin and $PGE_2$. Occasional specimens were accidentally destroyed or inadequate reducing the number of paired comparisons. There were no significant differences for any of the qualitative urine tests. No significant differences were observed for serum creatinine, potassium, bilirubin, GOT or GPT, all of which remained within normal limits.

Significant changes were noted in plasma cortisol levels (Table III). The mean pre-induction figures were not significantly different in the three groups. With oxytocin there was a definite rise during labour with a fall after delivery to below the initial level. With $PGF_{2\alpha}$ no significant changes occurred, but the responses were in the same direction as with oxytocin. Very little rise in plasma cortisol levels occurred during labour with $PGE_2$ but the postpartum figure was considerably lower than the initial one.

TABLE III

*Plasma cortisol levels*

| | Plasma cortisol levels (μg./100 ml.) | | |
|---|---|---|---|
| | Oxytocin | $PGE_2$ | $PGF_{2\alpha}$ |
| "Pre-induction" | | | |
| Mean | 37·45 | 39·67 | 40·58 |
| SD | 8·81 | 12·48 | 11·50 |
| "During infusion" | | | |
| Mean | 48·91(*a*) | 42·56 | 46·42 |
| SD | 23·04 | 18·12 | 19·33 |
| "Post delivery" | | | |
| Mean | 31 86(*b*) | 29·14(*c*) | 35 13 |
| SD | 9·05 | 7·82 | 14·69 |
| No. of patients in group | 21 | 24 | 19 |

(*a*) Significantly different from "pre-induction" value, $P<0 \cdot 01$, SE of difference, 3·93.

(*b*) Significantly different from "pre-induction" value, $P<0 \cdot 01$, SE of difference, 1·93.

(*c*) Very significantly different from "pre-induction" value, $P<0 \cdot 001$, SE of difference, 3·17.

Other differences were not significant, $P>0 \cdot 05$.

The mean serum sodium concentration was similar in all three groups before infusion commenced. There was no constant pattern to sodium concentrations during the period of observation for $PGF_{2\alpha}$ or $PGE_2$, but they fell during oxytocin infusion. The mean serum chloride level before infusion was lowest in the $PGE_2$ group and significantly different from the level in the other groups ($P<0 \cdot 05$). The serum chloride concentration fell during infusion and remained low in the oxytocin and $PGF_{2\alpha}$ groups, and the fall in

TABLE IV

*Change in concentration of serum sodium and chloride*

| | | Sodium (mEq./l.) | | | Chloride (mEq./l.) | | |
|---|---|---|---|---|---|---|---|
| | | Oxytocin | $PGE_2$ | $PGF_{2\alpha}$ | Oxytocin | $PGE_2$ | $PGF_{2\alpha}$ |
| "During infusion" level compared to "pre-induction" level | Mean change | −1·417 | −1·609 | −1·000 | −3·500 | −1·652 | −2·500 |
| | SE of change | 0·593 | 0·850 | 0·668 | 0·814 | 0·710 | 0·568 |
| | N | 24 | 23 | 24 | 24 | 23 | 24 |
| | P | <0·05 | n.s. | n.s. | <0·001 | <0·05 | <0·001 |
| "Post delivery" level compared to "pre-induction" level | Mean change | −0·625 | −0·174 | −1·041 | −2·375 | −1·087 | −2·416 |
| | SE of change | 0·639 | 0·608 | 0·720 | 0·727 | 0·697 | 0·787 |
| | N | 24 | 23 | 24 | 24 | 23 | 24 |
| | P | n.s. | n.s. | n.s. | <0·005 | n.s. | <0·01 |

n.s. = not significant or $P>0\cdot05$.

chloride was greater than the fall in sodium concentration especially for oxytocin. These results are probably related to the development of ketonuria (Table I).

An estimate was made of water balance during the infusion period. The average rate of gain or loss in ml./minute was calculated by subtracting the volumes of urine and any vomit from the combined oral and intravenous fluid intake and dividing by the total time of the collection. No allowance was made for losses from the skin and lungs. The results are shown in Table V. The mean water retention for oxytocin was higher than for either prostaglandin and the difference was significant in the case of $PGF_{2\alpha}$. For oxytocin there was a significant correlation ($r = 0\cdot494$; $P<0\cdot05$) between water retention and dose used. No such correlation existed for $PGE_2$ and $PGF_{2\alpha}$ ($r = 0\cdot145$ and $-0\cdot166$ respectively).

TABLE V

*Mean rate of water retention (ignoring skin and lung losses) in the interval between onset of infusion and delivery of placenta*

| | Oxytocin | $PGE_2$ | $PGF_{2\alpha}$ |
|---|---|---|---|
| Mean rate (ml./minute) | 1·193 | 0·789 | 0·664 |
| SD (ml./minute) | 0·851 | 0·932 | 0·613 |
| N | 24 | 23 | 25 |

Oxytocin vs. $PGE_2$ : $t=1\cdot554$, $P>0\cdot05$.
Oxytocin vs. $PGF_{2\alpha}$ : $t=2\cdot502$, $0\cdot01<P<0\cdot02$.

An estimate was made of sodium balance during infusion. Oral sodium intake was negligible but allowances were made for any intravenous saline given. As patients had different sodium intakes prior to induction, the baseline rate of excretion of sodium was calculated from the "pre-induction" urine specimen. This rate was deducted from the rate of sodium excretion for each urine collected during the infusion period. The net total gain and loss of sodium were then compared for the whole infusion period. The sodium concentration of vomit was arbitrarily assumed to be 70 mEq./l. There was a small positive sodium balance for all three agents; for oxytocin, the mean figure was 6·7 mEq. (SE = 9·5; n = 21), for $PGE_2$ it was 24·2 mEq. (SE = 18·4; n = 19) and for $PGF_{2\alpha}$ it was 8·4 mEq. (SE = 6·9; n = 24).

Changes in urine osmolalities during infusion were assessed by comparing the osmolality of the "pre-infusion" urine sample with that collected during the first four hours of infusion.

As indicated in Table VI there was a tendency

TABLE VI

*Changes in urine osmolality*

| | Osmolality of "pre-induction" urine compared to osmolality of urine collected during first 4 hours of infusion (mosm./kg.) | | |
|---|---|---|---|
| | Oxytocin | $PGE_2$ | $PGF_{2\alpha}$ |
| Mean change | −18·3 | −101·0 | −170·1 |
| SE | 38·6 | 59·3 | 45·6 |
| N | 22 | 19 | 23 |

The change for $PGF_{2\alpha}$ is significantly different ($P<0\cdot05$) from the change observed for oxytocin.

for urine osmolality to fall during infusion. The fall was greater for the prostaglandins. The mean volumes of fluid given intravenously during the induction were 400 and 370 ml. for $PGE_2$ and $PGF_{2\alpha}$ respectively, but for oxytocin the volume was somewhat greater at 625 ml. Despite the extra fluid load the fall was least for oxytocin.

There was no significant effect on plasma potassium concentration or on potassium excretion.

The other results of interest concern serum alkaline phosphatase levels. These were higher than in non-pregnant patients. Occasionally values in excess of 35 KA units/100 ml. were seen but were equally distributed among the three groups. The changes during and after labour were assessed by comparing values for each patient with their own pre-infusion figure. They show (Table VII) an insignificant rise during induction and a fall after delivery in all three groups. There was no difference between groups at the three times of measurement.

## DISCUSSION

The doses of the three agents used to induce labour were selected as those likely to be of equivalent biological activity. The patients were allocated at random but Table I suggests that those receiving $PGF_{2\alpha}$ had a lower mean parity and a rather longer labour.

Of the many different biochemical and haematological features studied, differences in behaviour between the different agents infused were mainly related to sodium, chloride and water balance and to adrenocortical responses as assessed by plasma cortisol levels. The lack of any change in platelet sensitivity to ADP during induced labour contrasts with a marked increase in such sensitivity after parturition (Sanderson *et al.*, 1973), and would suggest that there is no thrombogenic risk attendant in the use of any of the oxytocic agents studied.

The results indicate that oxytocin caused water retention without altering sodium balance. This was associated with a significant fall in serum sodium concentration and a relatively high urinary osmolality. No such effects were demonstrated with either prostaglandin. This is in agreement with the findings of Roberts *et al.* (1970) who found no impairment of water excretion even during induced diuresis when using $PGF_{2\alpha}$; their patients were in the second trimester and were given 0·05 μg./kg./minute. Our maximum dose of $PGF_{2\alpha}$ was 10 μg./minute. Water retention with oxytocin has been demonstrated in both pregnant and non-pregnant subjects (Abdul-Karin and Assali, 1961; Liggins 1962) and Whalley and Pritchard (1963) pointed out the risks of water intoxication with dilute solutions of oxytocin. There are obvious advantages in avoiding such effects in the induction of labour in patients with renal disease or pre-eclampsia.

Neither $PGE_2$ nor $PGF_{2\alpha}$ affected sodium excretion. Prostaglandins PGA and $PGA_2$ in

TABLE VII

*Changes in serum alkaline phosphatase*

| | | Changes in serum alkaline phosphatase levels (KA units/100 ml.) | | |
|---|---|---|---|---|
| | | Oxytocin | $PGE_2$ | $PGF_{2\alpha}$ |
| "During infusion" level compared to "pre-induction" level | Mean change | +0·167 | +0·857 | +0·818 |
| | SE | 0·759 | 0·626 | 0·737 |
| | N | 18 | 21 | 22 |
| "Post delivery" level compared to "pre-induction" level | Mean change | −3·167 | −2·619* | −2·091† |
| | SE | 1·731 | 0·615 | 1·095 |
| | N | 18 | 21 | 22 |

* P<0·001 (t = 6·70) for change, "during infusion" vs. "post delivery".
† P<0·05 (t = 2·31) for change, "during infusion" vs. "post delivery"

higher doses than those used in our study exerted a natriuretic effect particularly in hypertensive or oedematous patients (Lee *et al.*, 1971; Hornych *et al.*, 1972) but differences in biological activities between prostaglandins of different series are well recognized. There seems to be no positive advantage in using $PGE_2$ and $PGF_{2\alpha}$ in the induction of labour in oedematous patients at term in so far as sodium handling is concerned.

The changes in adrenocortical function are of interest. In all three groups plasma cortisol "pre-induction" figures were high, partly due to increased cortisol-binding globulin. However, the unfamiliar surroundings and procedures probably had a stressful effect. A further rise occurred during the infusion particularly with oxytocin, at a time of day when plasma cortisol normally falls. Although certain prostaglandins including $PGE_2$ have been shown to increase steroid production in isolated rat adrenals (Flack *et al.*, 1969), the only significant change in cortisol levels was seen in patients given oxytocin.

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# EFFECT OF FRUSEMIDE ON FETAL URINE PRODUCTION

BY

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## Summary

Hourly fetal urinary production rate (HFUPR) was measured by an ultrasonic technique in 12 antenatal patients before and after intravenous administration of 60 mg. of frusemide. In 8 patients pregnancy was normal and in 4 there was clinical evidence of fetal growth retardation. The duration of pregnancy varied from 32 to 41 weeks. The increase in HFUPR was 80 to 150 per cent in normal patients and 95 to 131 per cent in patients with fetal growth retardation. The increase in HFUPR seemed to have no impact on fetal circulatory volume, as the fetal heart rate stayed normal throughout the study period.

CAMPBELL *et al.* (1973) recently developed an ultrasonic technique of measuring hourly fetal urine production rates (HFUPR) in the human fetus. We were interested to know whether frusemide when given intravenously to the pregnant patient, would also have a diuretic effect on the fetus.

## METHODS

A total of 12 primigravidae between 32 and 41 weeks of pregnancy was screened (Table I). Each patient was certain of her last menstrual period. In 8 patients pregnancy was uncomplicated and in 4 there was clinical evidence of fetal growth retardation. In the latter group one patient had a slightly raised blood pressure (145/90 mm. Hg). None of the patients had oedema. There was no evidence of impaired maternal renal function. The blood creatinine (72 to 108 $\mu$mol./l.) and blood uric acid levels (0·13 to 0·36 mmol./l.) were within normal limits and there was no proteinuria.

In the 8 uncomplicated cases, the birth weights were above the 10th centile; in the 4 complicated cases they were twice between the 5th and 10th centile and twice below the 5th centile according to the tables of Thomson *et al.* (1968), corrections being made for fetal sex. The fetal bladder can be demonstrated on an ultrasonic B mode display. It can be observed to fill and empty at regular intervals (Campbell, 1972). Measurement of the HFUPR involves measuring the fetal bladder in three dimensions and calculating the volume by means of a formula (Campbell *et al.*, 1973). The increase in bladder volume over a known period during the process of bladder filling will give the HFUPR. In our study, bladder scanning was performed at different times of the day, as Wladimiroff and Campbell (1974) found no diurnal variation in HFUPR.

In the present study, the following procedure was carried out on each patient:

1. Measurement of the HFUPR before frusemide administration.
2. Intravenous administration of 60 mg. of frusemide into a maternal antecubital vein immediately after the fetal bladder had emptied.
3. Repeated measurements of fetal bladder volume during the filling phase after frusemide administration. These measurements were done at five minute intervals in order to ascertain the onset of any change in HFUPR due to frusemide.
4. Measurement of the amount of urine passed by each patient over a period of 90 minutes following frusemide administration.

TABLE I

*Fetal urinary output, maternal arterial blood pressure and fetal heart rate before and after intravenous frusemide administration in 12 antenatal patients between 32 and 41 weeks' gestation*

| BEFORE FRUSEMIDE ADMINISTRATION | | | | | | | AFTER FRUSEMIDE ADMINISTRATION | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient No. | Maturity (weeks) | HFUPR (ml.) | Blood pressure (mm. Hg) | Fetal heart rate range (beats per minute) | Time interval (minutes) | | Maximal HFUPR | | Final HFUPR (ml.) | Blood pressure range (mm. Hg) | Fetal heart rate range per (per minute) | Birth-weight centile (Thomson *et al.*, 1968) |
| | | | | | A–B* | B–C† | Actual rate (ml.) | increase (per cent) | | | | |
| 1 | 32 | 12·5 | 110/70 | 140–148 | 40 | 50 | 24·0 | 92 | 12·0 | 115/75–110/65 | 134–146 | >10 |
| 2 | 32 | 12·0 | 120/75 | 128–132 | 35 | 65 | 28·0 | 133 | 11·0 | 120/80–115/80 | 132–140 | >10 |
| 3 | 33 | 11·0 | 120/70 | 140–150 | 30 | 55 | 20·0 | 82 | 11·0 | 130/80–115/70 | 140–150 | >10 |
| 4 | 34 | 15·0 | 125/80 | 136–144 | 30 | 45 | 36·0 | 140 | 16·0 | 115/80 | 140–148 | >10 |
| 5 | 36 | 20·0 | 125/80 | 150–156 | 25 | 60 | 36·0 | 80 | 20·0 | 125/80–115/75 | 136–148 | >10 |
| 6 | 37 | 20·0 | 120/80 | 134–142 | 15 | 50 | 48·0 | 140 | 18·5 | 120/80 | 130–146 | >10 |
| 7 | 38 | 20·0 | 120/75 | 140–145 | 15 | 55 | 50·0 | 150 | 21·0 | 120/80–110/70 | 140–150 | >10 |
| 8 | 40 | 25·0 | 115/75 | 140–150 | 15 | 55 | 50·0 | 100 | 23·0 | 120/75–110/70 | 140–150 | >10 |
| 9 | 39 | 18·0 | 130/75 | 144–154 | 25 | 50 | 38·0 | 111 | 16·0 | 130/70–120/70 | 140–154 | 5–10 |
| 10 | 39 | 19·0 | 125/80 | 138–146 | 25 | 50 | 44·0 | 131 | 19·0 | 130/80–115/75 | 136–146 | 5–10 |
| 11 | 37 | 11·0 | 130/80 | 120–128 | 30 | 60 | 21·5 | 95 | 11·0 | 130/85–130/80 | 120–132 | <5 |
| 12 | 39 | 15·0 | 145/90 | 132–140 | 30 | 75 | 33·0 | 120 | 14·0 | 145/90–130/85 | 136–148 | <5 |

*Time interval A–B = delay between moment of frusemide administration and initial rise in HFUPR.

†Time interval B–C = duration of diuretic action of frusemide on the fetal kidneys.

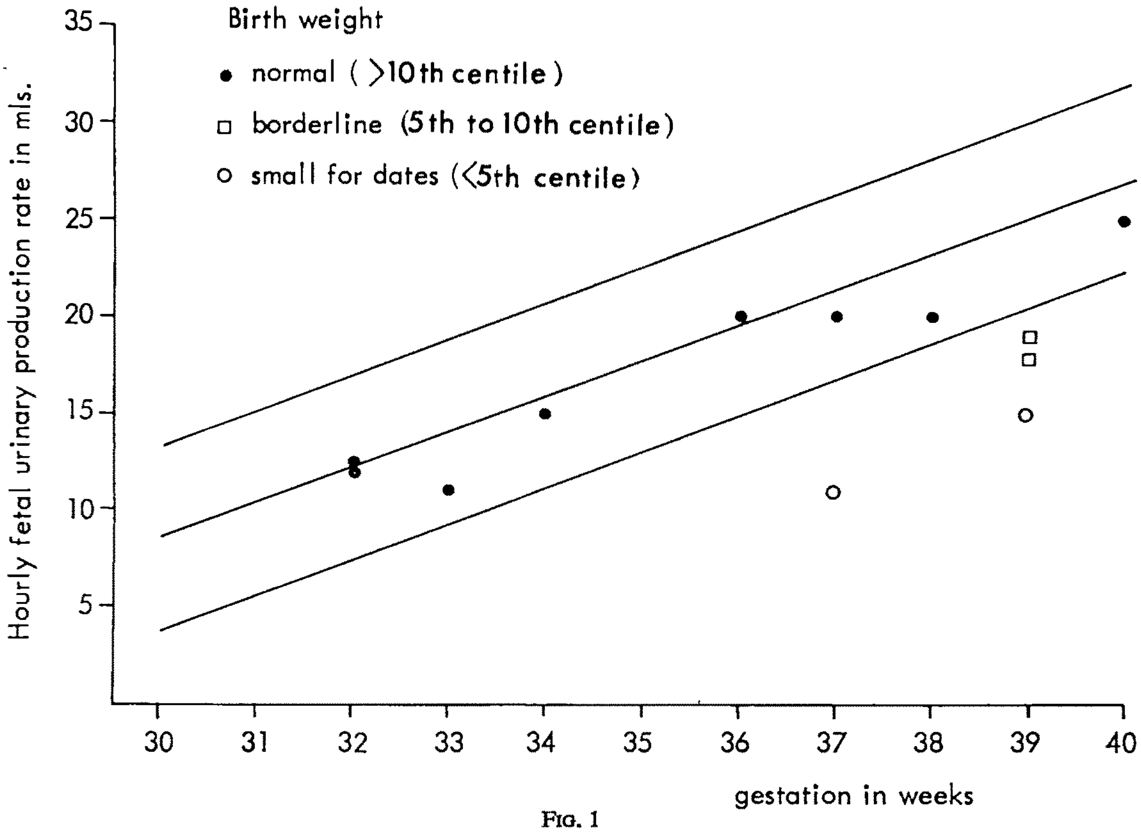

FIG. 1

Relation of the HFUPR estimations to the normal range (according to the data of Wladimiroff and Campbell, 1974) before frusemide administration.

5. Measurement of maternal arterial blood pressure in the supine position, once before and every 10 minutes after frusemide administration.
6. Monitoring the fetal heart rate (FHR) by means of Doppler ultrasound before and after frusemide administration.

## RESULTS

Before frusemide administration, HFUPR values ranged from 11 to 20 ml. Figure 1 shows the relation of the HFUPR estimations to the normal range according to the data of Wladimiroff and Campbell (1974). In the 8 normal pregnancies HFUPR was within the normal range; in the 4 complicated pregnancies HFUPR was below the normal range. Intravenous administration of 60 mg. of frusemide to the mother caused an increase in fetal urine production in all cases. Full details are given in Table I.

### *Normal pregnancies (patients 1 to 8)*

There was a delay between the moment of frusemide administration and the initial rise in HFUPR which ranged from 40 minutes at 32 weeks to 15 minutes at 40 weeks gestation (time interval A to B, Table I). The percentage increase in HFUPR ranged from 80 to 150 per cent. No relation to gestational age could be shown.

In all 8 patients HFUPR values finally returned to the original levels, as shown in Table I. No further decrease in HFUPR was observed. The total duration of diuretic action of frusemide on the fetal kidneys, (defined as the time between initial rise and final decrease of the HFUPR to original levels), ranged from 45 to 65 minutes (time interval B to C, Table I).

*Complicated pregnancies (patients 9 to 12)*

In the 4 complicated pregnancies the interval between the moment of frusemide administration and the initial rise in HFUPR ranged from 25 to 30 minutes.

The percentage increase in HFUPR ranged from 92 to 130 per cent. In all 4 patients, HFUPR finally returned to the original levels and no further decrease in HFUPR was observed. The total duration of the diuretic action of frusemide on the fetal kidneys ranged from 50 to 75 minutes.

Maternal urinary output varied from 850 to 1300 ml.

No significant changes in maternal arterial blood pressure and fetal heart rate after frusemide administration were observed in any of the 12 patients.

## DISCUSSION

Frusemide is a benzothiazide derivate which in the adult inhibits sodium reabsorption in the ascending limb of Henle's loop and in the proximal tubules (Stason *et al.*, 1966). Frusemide crosses the placental barrier (Pecorari *et al.*, 1969). The raised HFUPR estimations after intravenous administration of frusemide to the mother is the first direct proof of the presence of fetal tubular reabsorption of water and electrolytes during the last eight weeks of gestation.

In normal adults (Stason *et al.*, 1966) the delay between the moment of intravenous frusemide administration and the onset of its diuretic action is about five minutes. In our fetal studies the delay was longer. It varied from 40 minutes at 32 weeks to 15 minutes at 40 weeks gestation. This could be explained by the fact that the placental passage for frusemide becomes easier as pregnancy advances. The increased delay (25 to 30 minutes) in the 4 patients with clinical evidence of fetal growth-retardation when compared with the 3 normal patients (15 minutes, no. 6 to 8) in the last four weeks of pregnancy could have been caused by a hampered placental passage due to placental insufficiency. The duration of frusemide activity in the normal and growth-retarded fetuses did not apparently differ.

The percentage increase in fetal urinary output following the intravenous administration of 60 mg. frusemide varied considerably. Stason *et al.* (1966) who made similar observations on normal adults, stated that the variation in urinary output was a direct result of individuality in the response of tubules to frusemide. Whether the same applies to fetal tubules is uncertain as the amount of frusemide ultimately reaching the fetal kidneys was unknown. However, as the percentage increases in HFUPR in the normal patients and those with a growth-retarded fetus was similar, it is possible that fetal tubular function is essentially the same in these two groups. This would support the theory that the reduced HFUPR in growth-retarded fetuses is due to fetal renal hypoplasia (Wladimiroff and Campbell, 1974), rather than increased fetal tubular action as found in sheep (Gresham *et al.*, 1972).

Finally, the increase in HFUPR after frusemide apparently had no gross impact on fetal circulatory volume, as the fetal heart rate stayed normal throughout the study period.

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# PLACENTAL CHANGES IN PATIENTS WITH A LOW HAEMATOCRIT

BY

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## Summary

The relationship between haematocrit, macroscopic and histological features of the placenta and fetal weight were studied in a group of 50 Nigerian patients; half of them had a low haematocrit at booking. The presence of a low haematocrit (28 per cent or less) was associated with higher placental weight, lower fetal weight, better fetal/placental weight correlation and more villous fibrosis than were found in control patients with a haematocrit of 35 per cent or more.

IN Nigeria, the two important factors responsible for anaemia in pregnancy are folate deficiency and haemolysis related to malaria (Lawson, 1962; Fullerton and Turner, 1962; Fleming and Elliott, 1964; Ojo, 1965).

Although placental hypertrophy has been associated with maternal anaemia by some (Beischer *et al.*, 1970) there are no reports of histological study of the placenta in pregnancy anaemia. It is not known whether such a placenta exhibits any distinct or abnormal cellular features.

## MATERIALS AND METHODS

Fifty pregnant women were studied; 25 of them had a haematocrit of 28 per cent or less at booking (the anaemic group) and 25 had a haematocrit of 35 per cent or more (controls). All patients were given 300 mg. of ferrous gluconate three times daily, 5 mg. of folic acid once a day and 25 mg. of pyrimethamine (an antimalarial drug) once a week. Five women in the anaemia group still had a haematocrit of less than 28 per cent at delivery.

The haemoglobin genotype of each patient was determined by paper electrophoresis.

As soon as the placenta was delivered, the umbilical cord was cut flush with the placental surface and the membranes beyond the placental edge were trimmed with scissors. As much blood as possible was drained from the placenta which was then washed carefully in water to remove any adherent blood clots. The placenta was then weighed and afterwards placed in a jar containing 10 per cent formalin. The interval between delivery of the placenta and its immersion in formalin did not exceed 15 minutes. After several days, blocks of tissue about 1 cm. thick were cut from the central area of every quadrant of each placenta. Sections from each block were stained with haematoxylin and eosin. A hundred terminal villi in the maternal sub-division of each block were examined by light microscopy and thus a total of 400 villi were examined in each placenta (Fox, 1964). The number of villi showing syncytial knots, infarction, fibrosis, Hofbauer cells, oedema, nucleated erythrocytes and non-sinusoidal capillaries was noted. An earlier study in which Van Gieson's stain and haematoxylin and eosin were compared had shown that it was not difficult to identify fibrotic villi using haematoxylin and eosin and only villi which showed a gross excess of collagen were regarded as fibrotic.

The fetus was weighed within two hours of delivery.

TABLE I

*Non-histological comparisons between anaemic patients and controls. Only statistically significant differences between groups are indicated*

| | Mean duration of gestation | No. of patients with haemoglobin type | | | | Placental weight (g.) | | Fetal weight (g.) | | Mean placental/fetal weight ratio | Placental/fetal weight correlation | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | AA | AS | SS | SC | Mean | SD | Mean | SD | | r | P value |
| Anaemic group | 39·16 | 16 | 7 | 1 | 1 | 563·0 | 102·5 | 3107·7 | 381·3 | 0·181 | 0·47 | <0·02 |
| Control group | 38·96 | 16 | 9 | 0 | 0 | 507·5 | 75·6 | 3202·4 | 437·0 | 0·158 | 0·31 | >0·1 |
| | | | | | | p<0·05 | | | | | | |

TABLE II

*Comparison of placental histological features (mean percentage of 100 villi). Only statistically significant differences are indicated*

| | Incidence of histological features noted in 400 villi studied for each patient (per cent) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Syncytial knots | Villous fibrosis | Oedema | Hofbauer cells | Nucleated erythrocytes | Infarction without fibrosis | Non-sinusoidal vessels | |
| | | | | | | | Mean | SD |
| Anaemic group | 20·56 | 3·20 | 0·40 | 0·28 | 2·52 | 10·16 | 5·16 | 2·04 |
| Control group | 20·52 | 0·96 | 0·28 | 0·20 | 2·68 | 10·08 | 3·88 | 2·04 |
| | | p<0·01 | | | | | | |

## RESULTS

Table I summarizes the duration of gestation, haemoglobin type, placental weight, fetal weight, the placental/fetal weight coefficients, and placental/fetal weight correlations in the two groups of patients.

The histological features noted in the two groups are summarized in Table II.

There was no perinatal loss in the anaemic group and one control patient had a stillbirth.

## DISCUSSION

Of all the histological features studied, the only significant one in pregnancy anaemia was villous fibrosis. Why villous fibrosis should occur in anaemia is not explained by differences in gestational age at delivery or haemoglobin genotype. It is unlikely that villous fibrosis in anaemia results from thrombosis of a fetal stem artery or reduction of blood flow from obliterative endarteritis of the fetal stem artery which has been noted in placentae from diabetic and pre-eclamptic women. Fox (1967) has suggested that villous fibrosis in general might be due to the presence of non-sinusoidal capillaries which was found to be the hallmark of postmature placenta. However, the incidence of villi with non-sinusoidal capillaries in the two groups was similar.

The incidence of syncytial knots found in this study was similar to the normal values given by Benirschke (1961) and Merril (1963) and there was no correlation between villous fibrosis and syncitial knot formation.

Maternal anaemia was associated with increased placental weight but there was no evidence of an associated increase in fetal weight although anaemic patients did show a statistically significant correlation between fetal and placental weight which was not noted in controls. It can thus be concluded that there is a direct relationship between maternal haematocrit and the normality of the placenta as judged by placental weight, fetal weight and cellular changes.

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# THE OBSTETRIC PERFORMANCE OF NIGERIAN PRIMIGRAVIDAE AGED 16 AND UNDER

BY

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## Summary

The obstetric performance of 105 primigravidae aged 16 or under who received inadequate antenatal care was compared with that of 95 primigravidae of the same age who had adequate antenatal care and with 100 primigravidae aged 22 years who also had adequate antenatal care. The increased incidence of pre-eclampsia and babies weighing under 2·5 kg. in teenage pregnancy was confirmed. The effects of both hazards were diminished by good antenatal care.

SINCE Harris's paper on the obstetric performance of the pregnant teenager was published in 1922 (Harris, 1922), several other reviews have been written on the subject mainly from the United States of America and Europe. Comparatively few papers have been written on the outcome of pregnancy in the Negro teenager adequately managed in tropical Africa, and the true risk of pregnancy in this group of patients still awaits elucidation. Scher and Utian (1970), in a multiracial study carried out in South Africa, found that, as compared with whites, the Bantu primigravida maintained a high fetal head till term and that the incidence of prematurity was higher in their Bantu patients than in their Cape coloured and their white patients. These authors, however, stated that their Bantu patients had inadequate antenatal care and came from a lower social class than their white patients.

Apart from prematurity, young primigravidae are also held to be more prone to pre-eclampsia, hypertension, anaemia, prolonged labour and postpartum haemorrhage by a number of authors (Claman and Bell, 1964; Wallace, 1965; Zackler *et al.*, 1969).

The present study was undertaken to assess the degree of these risks in Southern Nigerians and to determine how far adequate antenatal care might help in preventing these complications.

### METHODS AND RESULTS

The course and outcome of pregnancy was studied in two groups of primigravidae who were aged 16 years and under (hereinafter called teenagers). The first teenage group consisted of 105 patients who received inadequate antenatal care (as defined later) in various centres outside the Lagos University Teaching Hospital (LUTH) between January 1967 and December 1973, while the second teenage group consisted of 95 primigravidae who received adequate antenatal care (usually at the LUTH) during the same period. One hundred consecutive primigravidae aged 22 years given adequate antenatal care at the LUTH in 1972 and 1973 acted as controls. In the teenage group receiving inadequate antenatal care, 2 patients were aged 13, 9 were aged 14, 40 were aged 15, and 54 were aged 16; 42 (40 per cent) of patients in this group were married. In the group receiving adequate antenatal care, 5 patients were aged 13, 6 were aged 14, 36 were aged 15, and 48 were aged 16; 29 (31 per cent) of these patients were married. Hassan and Falls (1964) defined adequate antenatal care as consisting of five visits or more starting on or before the 13th week of pregnancy. All patients in the group with inadequate antenatal care attended their respective antenatal clinics less than five times and were often first seen in the second trimester. The remainder received adequate antenatal care.

*Social class*

Patients accepted for antenatal care and delivery at the LUTH are divided into five groups, according to their husbands' or fathers' annual income. In Group I are patients whose husbands or fathers earn more than 10 000 naira (about £5000) annually. The corresponding income figures for Groups II to V are respectively 5000 to 10 000 naira, 3000 to 5000 naira, 1000 to 3000 naira, and less than 1000 naira. In Nigeria, directors of big companies fall into Group I whereas most professional men such as medical or legal practitioners and University Professors are in Group II.

As shown in Table I, most of the patients belonged to the lower income groups. However, 18 per cent of the control group were wives or daughters of men in income Group II.

TABLE I
*Distribution of patients according to income group of husband or father*

| Income group | Teenagers given inadequate care (per cent) | Teenagers given adequate care (per cent) | Controls (per cent) |
|---|---|---|---|
| I | 0 | 0 | 3 |
| II | 2·1 | 5·1 | 18 |
| III | 3·6 | 0 | 6 |
| IV | 7·5 | 7·3 | 29 |
| V | 67·5 | 78·5 | 35 |
| Unknown | 19·3 | 9·1 | 9 |
| Total | 100·0<br>(n = 105) | 100·0<br>(n = 95) | 100<br>(n = 100) |

*Maternal height*

The maternal height distribution is shown in Table II.

TABLE II
*Maternal height distribution*

| Height (cm.) | Teenagers given inadequate antenatal care (per cent) | Teenagers given adequate antenatal care (per cent) | Controls (per cent) |
|---|---|---|---|
| 153 and under | 40 | 40 | 15 |
| 154 to 160 | 43 | 53·7 | 52 |
| 161 and over | 11·3 | 6·3 | 33 |
| Unknown | 5·7 | Nil | Nil |
| Total | 100 | 100 | 100 |

*Pre-eclampsia, eclampsia and other antenatal complications*

Mild pre-eclampsia was defined as an increase of 20 mm. Hg or more in the mean arterial pressure occurring with "more than a trace" of proteinuria in the second half of pregnancy. Severe pre-eclampsia was defined as a rise in the mean arterial pressure of 30 mm. Hg. or more with significant proteinuria. The incidence of mild or severe pre-eclampsia and eclampsia is shown in Table III which also records the incidence of syphilis, anaemia and malaria in the three groups.

All patients who had syphilis were treated as soon as the serological diagnosis was made and their babies were not affected.

*Labour*

The duration of pregnancy could not be estimated with any degree of accuracy as most of the patients were uncertain of their dates and so no information can be given about gestational age at the onset of labour. The duration of labour in all three groups of patients is shown in Table IV. Table V shows the mode of delivery; in spite of a slightly higher forceps delivery rate in the control group there were no statistical differences between the three groups. The blood loss at delivery is shown in Table VI and was similar for all groups.

*Infants*

The birthweight of infants in the three groups is shown in Table VII as is the maternal height.

TABLE III

*Incidence of antenatal complications*

| Complications | Teenagers given inadequate antenatal care | | Teenagers given adequate antenatal care | | Controls | |
|---|---|---|---|---|---|---|
| | Number | Per cent | Number | Per cent | Number | Per cent |
| Syphilis | Unknown | – | 7 | 7·4 | 0 | 0 |
| Pre-eclampsia | | | | | | |
| Mild | 4 | 3·8 | 12 | 12·6 | 3 | 3 |
| Severe | 7 | 6·7 | 4 | 4·2 | 0 | 0 |
| Eclampsia | 3 | 2·9 | 1* | 1·0 | 0 | 0 |
| Anaemia† | | | | | | |
| Iron deficiency | 37 | 35·2 | 21 | 22·1 | 45 | 45 |
| Sickle cell disease | 1 | 0·95 | 1 | 1·05 | 0 | 0 |
| Malaria | Unknown | – | 14 | 14·7 | 8 | 8 |

* Admitted as emergency from another institution and died shortly after arrival at University Hospital.

† Anaemia was defined as a haemoglobin concentration of 8·7 g./100 ml. or less (Lawson, 1967).

TABLE IV

*Duration of labour*

| Duration of labour (hours) | Teenagers given inadequate antenatal care | | Teenagers given adequate antenatal care | | Controls | |
|---|---|---|---|---|---|---|
| | Number | Per cent | Number | Per cent | Number | Per cent |
| Under 12 | 54 | 51·4 | 52 | 54·7 | 41 | 41 |
| 13 to 24 | 33 | 31·5 | 30 | 31·6 | 35 | 35 |
| Over 24 | 18 | 17·1 | 13 | 13·7 | 24 | 24 |
| Total | 105 | 100·0 | 95 | 100·0 | 100 | 100 |

TABLE V

*Method of delivery*

| Method of delivery | Teenagers given inadequate antenatal care | | Teenagers given adequate antenatal care | | Controls | |
|---|---|---|---|---|---|---|
| | Number | Per cent | Number | Per cent | Number | Per cent |
| Spontaneous | 94 | 89·5 | 81 | 85·3 | 81 | 81 |
| Breech | 0 | 0 | 2 | 2·1 | 1 | 1 |
| Forceps | 4 | 3·8 | 4 | 4·2 | 11 | 11 |
| Ventouse | 0 | 0 | 0 | 0 | 1 | 1 |
| Caesarean section | 7 | 6·7 | 8 | 8·4 | 6 | 6 |
| Total | 105 | 100·0 | 95 | 100·0 | 100 | 100 |

TABLE VI

*Blood loss at delivery*

| Blood loss (ml.) | Teenagers given inadequate antenatal care | | Teenagers given adequate antenatal care | | Control group | |
|---|---|---|---|---|---|---|
| | Number | Per cent | Number | Per cent | Number | Per cent |
| Under 300 | 98 | 93·3 | 88 | 92·6 | 84 | 84 |
| 300 to 600 | 6 | 5·7 | 5 | 5·3 | 15 | 15 |
| Over 600 | 1 | 1·0 | 2 | 2·1 | 1 | 1 |
| Total | 105 | 100·0 | 95 | 100·0 | 100 | 100 |

TABLE VII

*Birthweight and maternal height*

| Birthweight/range (kg.) | Teenagers given inadequate antenatal care | | Teenagers given adequate antenatal care | | Control group | |
|---|---|---|---|---|---|---|
| | Number | Per cent | Number | Per cent | Number | Per cent |
| 2·5 and less | 25* | 23·6 | 12* | 12·6 | 8 | 8 |
| 2·6 to 3·0 | 48 | 45·4 | 44 | 46·3 | 28 | 28 |
| 3·1 to 3·50 | 24 | 22 6 | 27 | 28·4 | 43 | 43 |
| 3·6 to 3·85 | 8 | 7·5 | 9 | 9·5 | 19 | 19 |
| Over 3·85 | 1 | 0·9 | 3 | 3·2 | 2 | 2 |
| Total | 106 | 100 | 95 | 100 | 100 | 100 |
| Birthweight (kg.) Mean | 2·6 | | 2·9 | | 3·1 | |
| SD | ±0 57 | | ±0·51 | | ±0·51 | |
| Range | 1·3–4·0 | | 1·9–3·9 | | 1·4–4·7 | |
| Maternal height (cm.) Mean | 154·6 | | 152·7 | | 159·6 | |
| SD | ±6·2 | | ±5·4 | | ±6·7 | |
| Range | 137–170·2 | | 140–167·6 | | 142·2–180·3 | |

* Significantly different.
($\chi^2 = 4{\cdot}38$; $p<0{\cdot}05$)

Ten of the twelve babies weighing less than 2·5 kg. born to patients given adequate antenatal care and seven of the eight similar control babies were clinically immature. There were nine stillbirths and five neonatal deaths in the teenage patients given inadequate care, no stillbirths and one neonatal death in the teenagers given adequate care, and one stillbirth and three neonatal deaths in the control group. One of the neonatal deaths in the control group was due to severe multiple congenital abnormalities.

## DISCUSSION

The present study suggests that the risks associated with small babies (birthweight less than 2·5 kg.) and pre-eclampsia which occur in teenage pregnancy are small and that their effects can be significantly reduced by good antenatal care. Roopnarinesingh (1970) found an increased incidence of both small babies and pre-eclampsia in 122 negro patients in the West Indies. He, however, also reported a higher incidence of instrumental delivery and postpartum haemorrhage which is at variance with our own findings. A more recent review by Hay and Boyd (1973) from Kingston, Jamaica, also pointed to an increased incidence of small babies but not of pre-eclampsia in young primigravidae. Wallace (1965) stressed the fact that small babies might not be an inherent factor in the obstetric performance of young primigravidae and held that their diet habits and social class might be responsible. In support of this view might be cited the reviews by Stearn (1963) and Obeng (1969) who analysed patients from the same social groups in London and found no increased incidence of small babies compared with controls. Our own findings point more directly to the patients' immediate obstetric care as determining the weight of her baby. Eight per cent of control mothers were delivered of infants weighing 2·5 kg. and under compared with 23·6 per cent of teenagers given inadequate antenatal care. This percentage was almost halved when the teenage primigravidae received adequate antenatal care (Table VII).

One explanation for the low incidence of small babies in the patients given adequate antenatal care could be the better control of malaria and anaemia in this group. The true incidence of malaria in the teenagers given inadequate antenatal care could not be determined but was thought to be much higher than in any of the other two groups because all patients given adequate antenatal care were given antimalarial prophylaxis routinely. Anaemia which was found to be more common in the patients with inadequate antenatal care could also have been due to repeated attacks of malaria. Most of the anaemic patients from all groups had iron deficiency anaemia. Sickle cell anaemia and sickle cell haemoglobin -C disease were the cause of anaemia in a very small percentage of patients (1 per cent) even in the teenage group given adequate antenatal care which colleagues referred early because of a known "sickle cell state". Hay and Boyd (1973) also found that "sickle cell state" accounted for only a small number (0·9 per cent) of their pregnant anaemic primigravidae in Kingston, Jamaica.

Pre-eclampsia was more common in both groups of teenage primigravidae than in the controls and this finding agreed with those of others. We were, however, surprised to discover a higher incidence of mild pre-eclampsia in the teenagers given adequate antenatal care than in those who had inadequate care (12·6 per cent and 3·8 per cent respectively). This may be due to the increased awareness of this condition in hospital practice. Severe pre-eclampsia and eclampsia were more common in the inadequately managed teenagers than in the other two groups. The only eclamptic patient in the teenage patients given adequate antenatal care was one who was seen outside the University hospital and had such a rapidly fulminating disease that she died within a few hours of admission to the University hospital. The fulminating nature of uncontrolled pre-eclampsia in young primigravidae was referred to by Utian (1957) reporting on South African whites.

Labour was comparatively short and safe in both groups of teenagers as compared with the control group despite the fact that the average height of the teenagers was less than that of the control patients. The rapidity of labour and the amount of blood loss did not bear a direct relationship with adequacy of antenatal care (Tables IV to VI). The Caesarean section rate was identical for all three groups but many more patients in the control group than in the teenage groups had forceps deliveries.

Failure of the fetal head to engage in the maternal pelvis around the 36th week of pregnancy was reported by Scher and Utian (1970) as a surprise finding in Bantu patients. In our own series engagement of the fetal head before the onset of labour was the exception rather than the rule and this is probably due to the high angle of pelvic inclination present in the negro.

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# THE EFFECT OF INTRAVENOUS RITODRINE ON THE ACID-BASE STATUS OF THE FETUS DURING THE SECOND STAGE OF LABOUR

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## Summary

A progressive fetal respiratory acidosis occurs in the second stage of labour. A double-blind controlled trial showed that the intravenous infusion of Ritodrine abolished this.

EVIDENCE exists that the second stage of labour is an asphyxial event for the fetus (Wood *et al.*, 1973*a* and *b*), and may harm a fetus already at risk.

"Ritodrine",* a β-mimetic drug, has been used successfully to treat established fetal distress in the first stage of labour (Renaud *et al.*, 1973*a*; Caldeyro-Barcia *et al.*, 1969) and when taken over some weeks it is associated with increased fetal weight (Seidel *et al.*, 1973). These effects are probably due to the improved materno-placental blood flow which seems to occur during uterine contractions in patients treated with Ritodrine (Cosmi and Condorelli, 1973; Renaud *et al.*, 1973*b*).

In an attempt to prevent fetal asphyxiation in the second stage of labour, we conducted a double-blind controlled trial of the effect of intravenous Ritodrine on fetal condition.

## MATERIALS AND METHODS

Thirty-five women, between 36 and 42 weeks gestation (with uncomplicated pregnancies), were studied in labour. Patients were specifically excluded from the trial if there were any clinical signs of fetal distress or if the mother had any significant cardiovascular disease.

As early as possible in the second stage of labour, a fetal scalp capillary blood sample was taken, the patient being deleted from the series if the pH of this sample was less than 7·20 (Lumley *et al.*, 1970).

At the same time an intravenous infusion was commenced, at a set rate (30 drops per minute), of either Ritodrine or placebo in 5 per cent dextrose. The Ritodrine content of the flask was such that the infusion was given at a dose rate of either 1·5 μg./kg./minute (low dose group) or 3·0 μg./kg./minute (high dose group). The placebo used was normal saline, in coded ampoules identical to those containing Ritodrine. The patients were randomly allocated to either the control group or to one of the treatment groups, and the contents of the intravenous flask were not known until after the patient had delivered and all measurements had been made. Thus a double-blind controlled trial was established.

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*Ritodrine HCl. Philips-Duphar N.V. Amsterdam, The Netherlands: D, L-erythro-1-(4-hydroxyphenyl)-2-(20-(40-hydroxyphenyl) ethylamino)-propanol hydrochloride.

The medical and nursing staff continued to manage the second stage of labour in the usual fashion: pethidine (100 to 200 mg.), and intermittent inhalation of a mixture of 60 per cent nitrous oxide and 40 per cent oxygen were used for pain relief, and all deliveries were performed in the supine position. As the mean duration of the second stage of labour is approximately 30 minutes, the staff were asked to proceed to a forceps delivery if a normal delivery was not imminent 30 minutes after the infusion was begun. Note was made of the times taken to deliver the child (Wood *et al.*, 1973*a*), of the mode of delivery, and of the presence or absence of any cord complications.

Prior to the onset of respiration, the umbilical cord was double clamped in two places and divided. Arterial and venous blood was then aspirated from the isolated cord segment. A specimen of "arterialized" maternal finger-tip capillary blood was also collected at delivery (Ang *et al.*, 1969).

After delivery, the infusion was discontinued, and 0·5 mg. of ergometrine administered intravenously.

All blood samples (fetal scalp capillary blood, umbilical arterial and venous blood, and maternal capillary blood) were collected in heparinized nylon capillary tubing, sealed from the air by alligator clips, stored at 0 to 4 °C., and examined within four hours of collection (Lumley *et al.*, 1971).

The blood samples were analyzed in duplicate for pH, $pO_2$ and $pCO_2$, using an Instrumentation Laboratories 213–03 digital blood-gas and pH analyzer. Oxygen saturation and base deficit at a haemoglobin of 5 g./100 ml. were calculated by the use of the appropriate nomograms (Siggaard-Anderson, 1963; Hellegers and Schruefer, 1961). The analyzer was calibrated for oxygen and carbon dioxide using four analyzed gas mixtures. The partial pressures of one of the gas mixtures were in the range of the values being determined in the blood samples. pH calibration was achieved with two commercially prepared standard buffers. The 95 per cent confidence limits for duplicate samples in this study were ±0·017 pH units, ±1·9 mm. Hg. $pO_2$± and ±2·3 mm. Hg. $pCO_2$.

The results were statistically evaluated by the appropriate application of the Student's "t" test, the F test, the chi-square test, the method of "least-squares" for obtaining lines of best fit, and Pearson's correlation coefficients (Armitage, 1971).

## RESULTS

### 1. *Analysis of groups for obstetric comparability*

The three patient groups were compared for maternal age and parity, fetal gestation, birthweight, initial fetal scalp pH, maternal capillary pH, presence or absence of cord complications, and mode of delivery (Table I). The only significant difference found was that the mean maternal capillary pH was lower in the group given the larger dose of Ritodrine.

Ritodrine increased the infusion to delivery interval (as close an approximation as can be made to the length of the second stage of labour) by more than 50 per cent. There was no significant difference in this effect between the two doses given. Ritodrine did not affect the time taken to deliver the fetus.

### 2. *Fetal condition*

Analysis of the correlation between the infusion-delivery interval and the cord blood measurements in the control group showed a progressive fall in pH, $pO_2$ and oxygen saturation, and a progressive rise in $pCO_2$. There was no change in base deficit (Figs. 1 to 5). In the Ritodrine groups these time-related changes were abolished and, in some cases, reversed (Figs. 1 to 5).

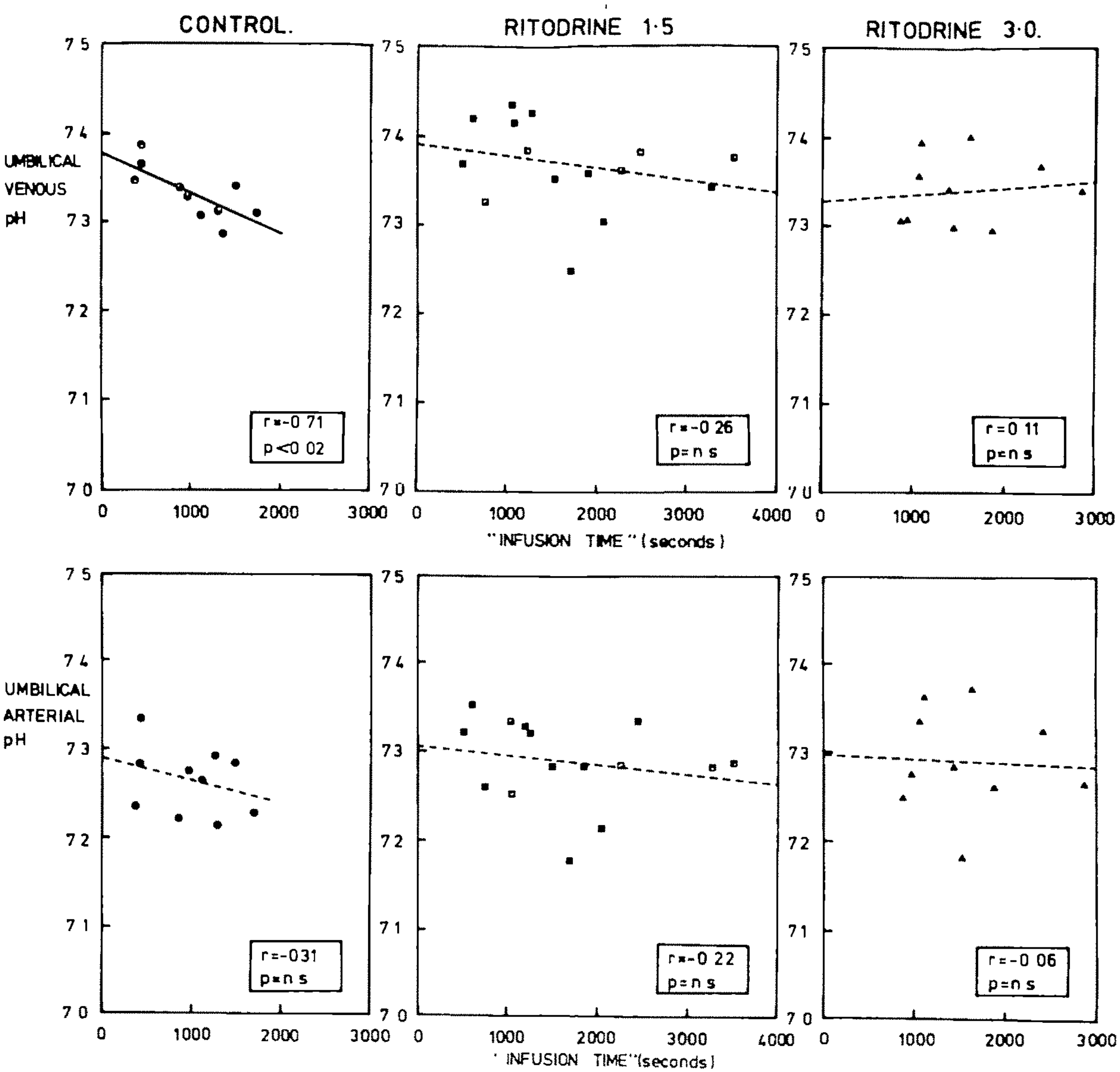
The results were also analyzed by dividing each group of patients into those with infusion-delivery intervals of less than, and greater than, 15 minutes (Table II). In the control group the patients who took longer to deliver showed a lower umbilical cord blood pH, $pO_2$ and oxygen saturation, and a higher $pCO_2$—these differences were significant for umbilical venous pH and $pCO_2$. In contrast the group given the small dose of Ritodrine did not show significant changes.

Comparison of the control and Ritodrine groups, considering fetuses delivered more than 15 minutes after starting the infusion, is shown in Table III. The pH, $pO_2$ and oxygen saturation

TABLE I

*Comparison of patient groups*

| | | Low dose Ritodrine (1·5 μg./kg./minute) | | Controls (placebo) | | High dose Ritodrine (3·0 μg./kg./minute) |
|---|---|---|---|---|---|---|
| Maternal age | mean±S.D. | 23·7±4·1 | | 24·3±6·6 | | 23·0±6·3 |
| | t | | 0·30 (p=n.s.) | | −0·20 (p=n.s.) | |
| | F | | 2·57 (p=n.s.) | | 0·63 (p=n.s.) | |
| Parity | mean±S.D. | 1·4±1·2 | | 1·4±1·1 | | 0·9±1·9 |
| | t | | 0·75 (p=n.s.) | | 0·74 (p=n.s.) | |
| | F | | 1·24 (p=n.s.) | | 0·34 (p=n.s.) | |
| Gestation (weeks) | mean±S.D. | 40·1±1·4 | | 39·3±1·2 | | 39·6±1·1 |
| | t | | −1·44 (p=n.s.) | | −0·60 (p=n.s.) | |
| | F | | 0·70 (p=n.s.) | | 1·16 (p=n.s.) | |
| Birthweight (kg.) | mean±S.D. | 3·52±0·42 | | 3·23±0·34 | | 3·34±0·56 |
| | t | | −1·78 (p=n.s.) | | −0·51 (p=n.s.) | |
| | F | | 0·66 (p=n.s.) | | 0·37 (p=n.s.) | |
| Total delivery time (seconds) | mean±S.D. | 99·3±38·9 | | 89·7±39·2 | | 75·2±41·8 |
| | t | | −0·60 (p=n.s.) | | 0·80 (p=n.s.) | |
| | F | | 1·01 (p=n.s.) | | 0·88 (p=n.s.) | |
| Infusion time (seconds) | mean±S.D. | 1653·3±906·7 | | 981·7±486·9 | | 1553·7±658·6 |
| | t | | −2·14 (p<0·05) | | −2·21 (p<0·05) | |
| | F | | 0·29 (p=n.s.) | | 0·54 (p=n.s.) | |
| | t | | | 0·30 (p=n.s.) | | |
| | F | | | 1·90 (p=n.s.) | | |
| pH fetal scalp blood | mean±S.D. | 7·326±0·045 | | 7·316±0·040 | | 7·323±0·027 |
| | t | | −0·58 (p=n.s.) | | −0·46 (p=n.s.) | |
| | F | | 0·82 (p=n.s.) | | 2·66 (p=n.s.) | |
| pH maternal blood | mean±S.D. | 7·401±0·051 | | 7·431±0·058 | | 7·377±0·043 |
| | t | | 1·33 (p=n.s.) | | 2·35 (p<0·05) | |
| | F | | 1·29 (p=n.s.) | | 1·82 (p=n.s.) | |
| Cord complications | present | 7 | | 2 | | 2 |
| | absent | 8 | | 8 | | 8 |
| | $\chi^2$ | | 1·01 (p=n.s.) | | 0·2 (p=n.s.) | |
| Mode of delivery | normal | 9 | | 8 | | 8 |
| | forceps | 6 | | 2 | | 2 |
| | $\chi^2$ | | 0·47 (p=n.s.) | | 0·2 (p=n.s.) | |



COMPARISON of
REGRESSION LINES

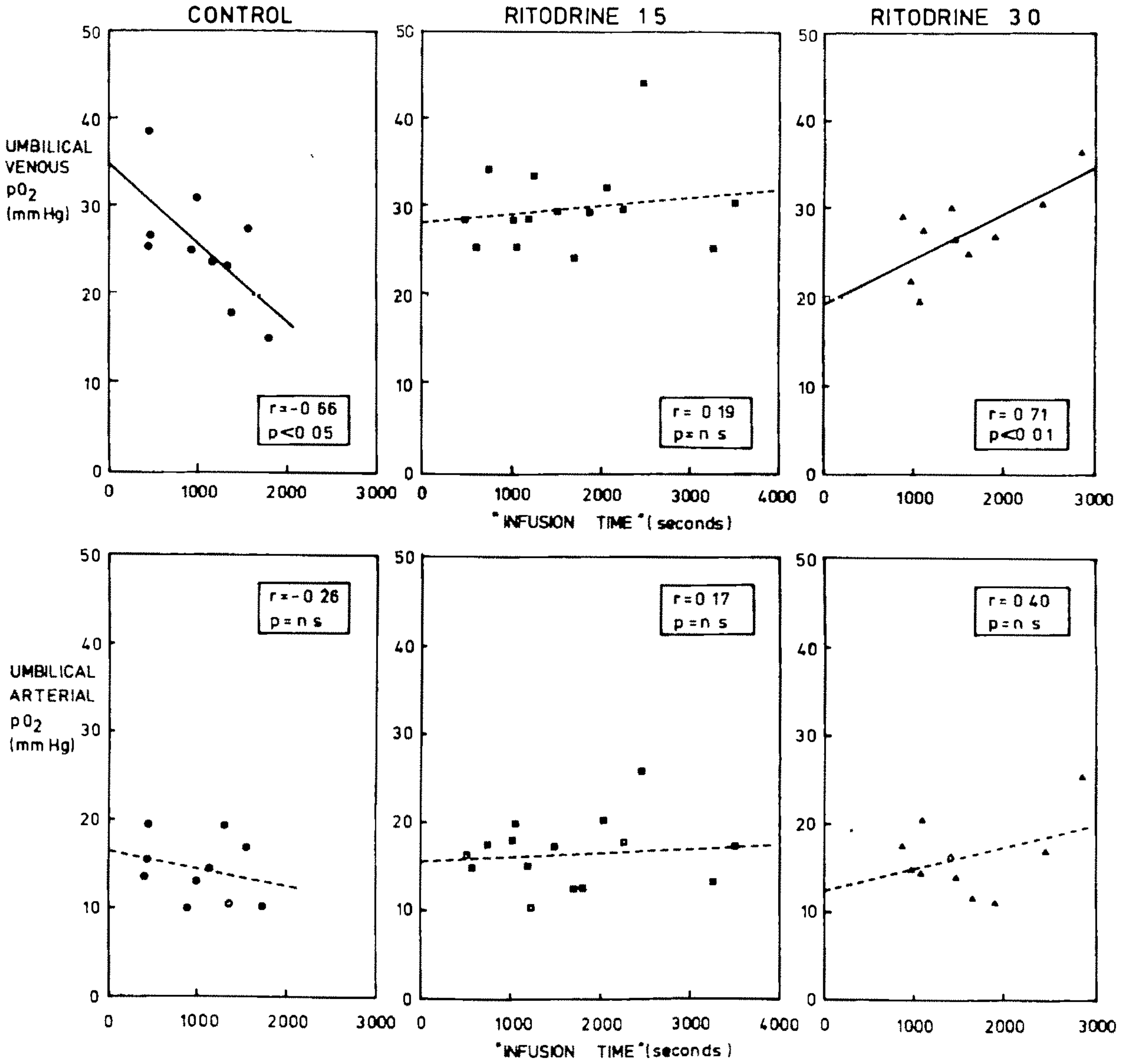
Control *vs.*
Ritodrine 1·5 μg./kg./minute
p<0·05

Control *vs.*
Ritodrine 3 0 μg./kg./minute
p<0·025

Control *vs.*
Ritodrine 1·5 μg./kg./minute
p=n.s.

Control *vs.*
Ritodrine 3·0 μg./kg./minute
p=n.s.

FIG. 1
Correlation of umbilical cord blood pH and "infusion time" (interval between start of infusion and delivery) in control and drug groups.



COMPARISON of REGRESSION LINES

Control *vs.*
Ritodrine 1·5 μg./kg./minute
p<0·02

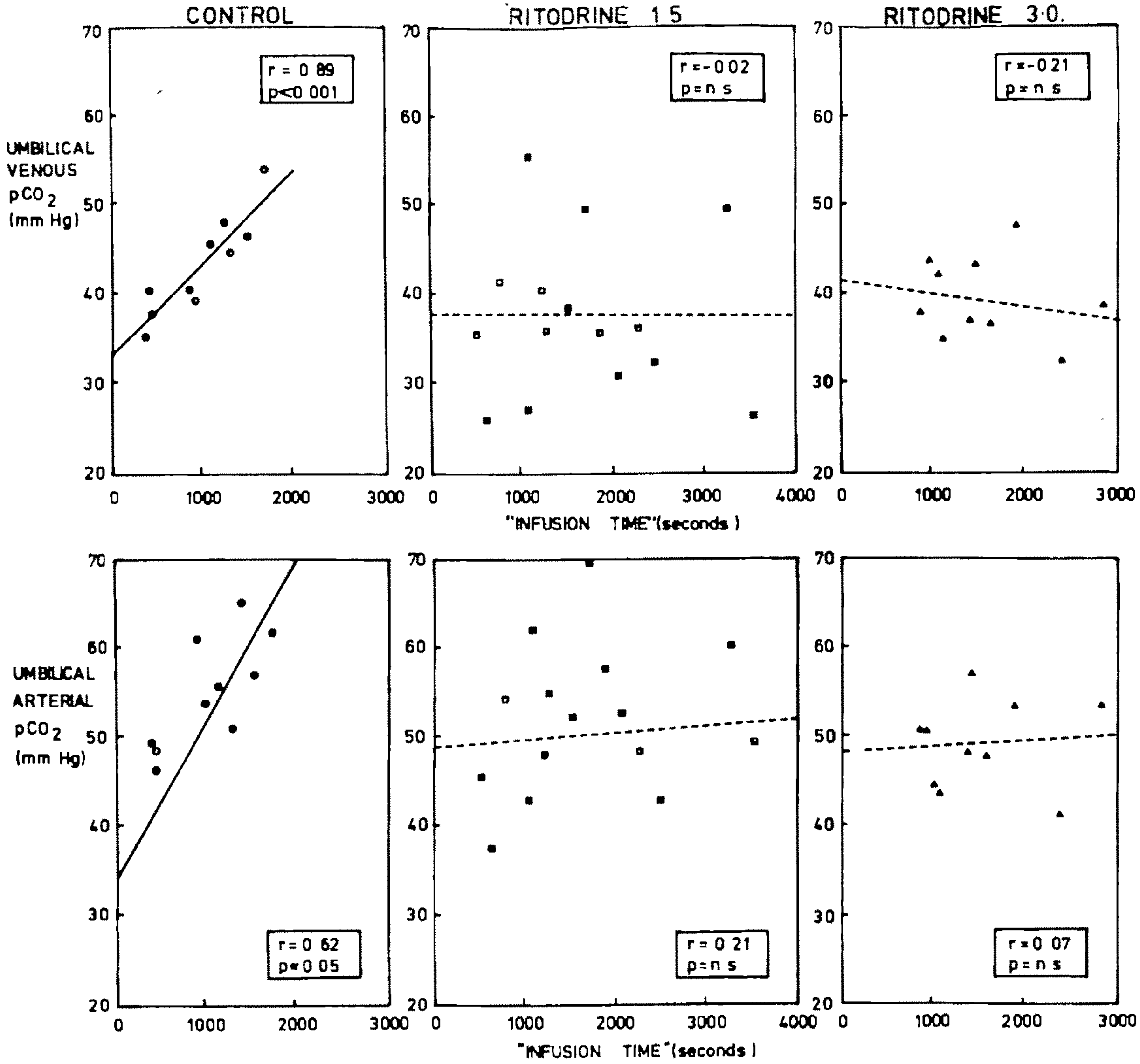
Control *vs.*
Ritodrine 3·0 μg./kg./minute
p<0·01

Control *vs.*
Ritodrine 1·5 μg./kg./minute
p=n.s.

Control *vs.*
Ritodrine 3·0 μg./kg./minute
p=n.s.

FIG. 2

Correlation of umbilical cord blood $pO_2$ and "infusion time" (interval between start of infusion and delivery) in control and drug groups.



COMPARISON of REGRESSION LINES

Control *vs.*
Ritodrine 1·5 μg./kg./minute
p=n.s.

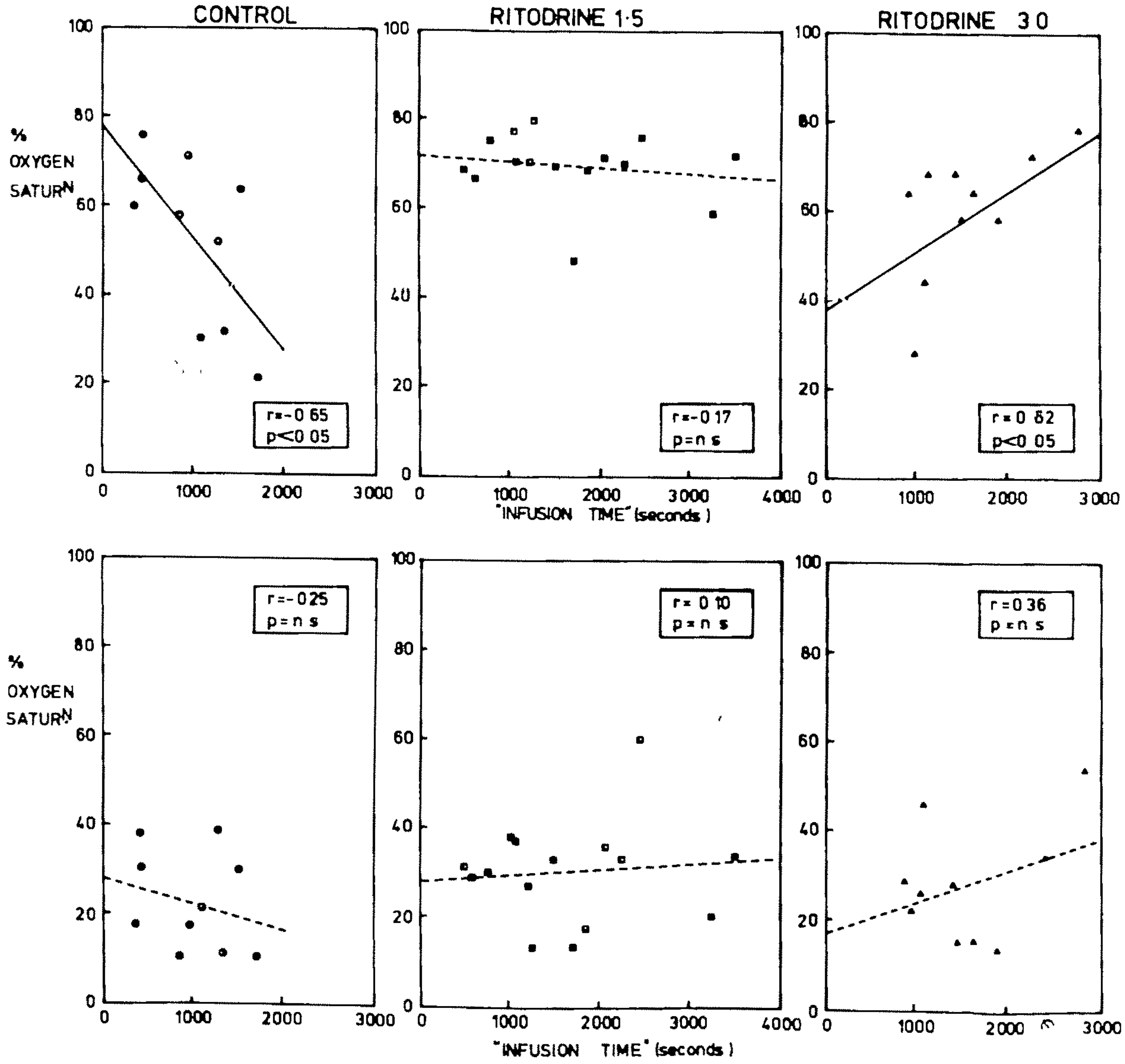
Control *vs.*
Ritodrine 3·0 μg./kg./minute
$p<0{\cdot}05$

Control *vs.*
Ritodrine 1·5 μg./kg./minute
$p<0{\cdot}05$

Control *vs.*
Ritodrine 3·0 μg./kg./minute
$p<0{\cdot}05$

FIG. 3

Correlation of umbilical cord blood $pCO_2$ and "infusion time" (interval between start of infusion and delivery) in control and drug groups.



COMPARISON of REGRESSION LINES

Control *vs.*
Ritodrine 1·5 μg./kg./minute
p<0·02

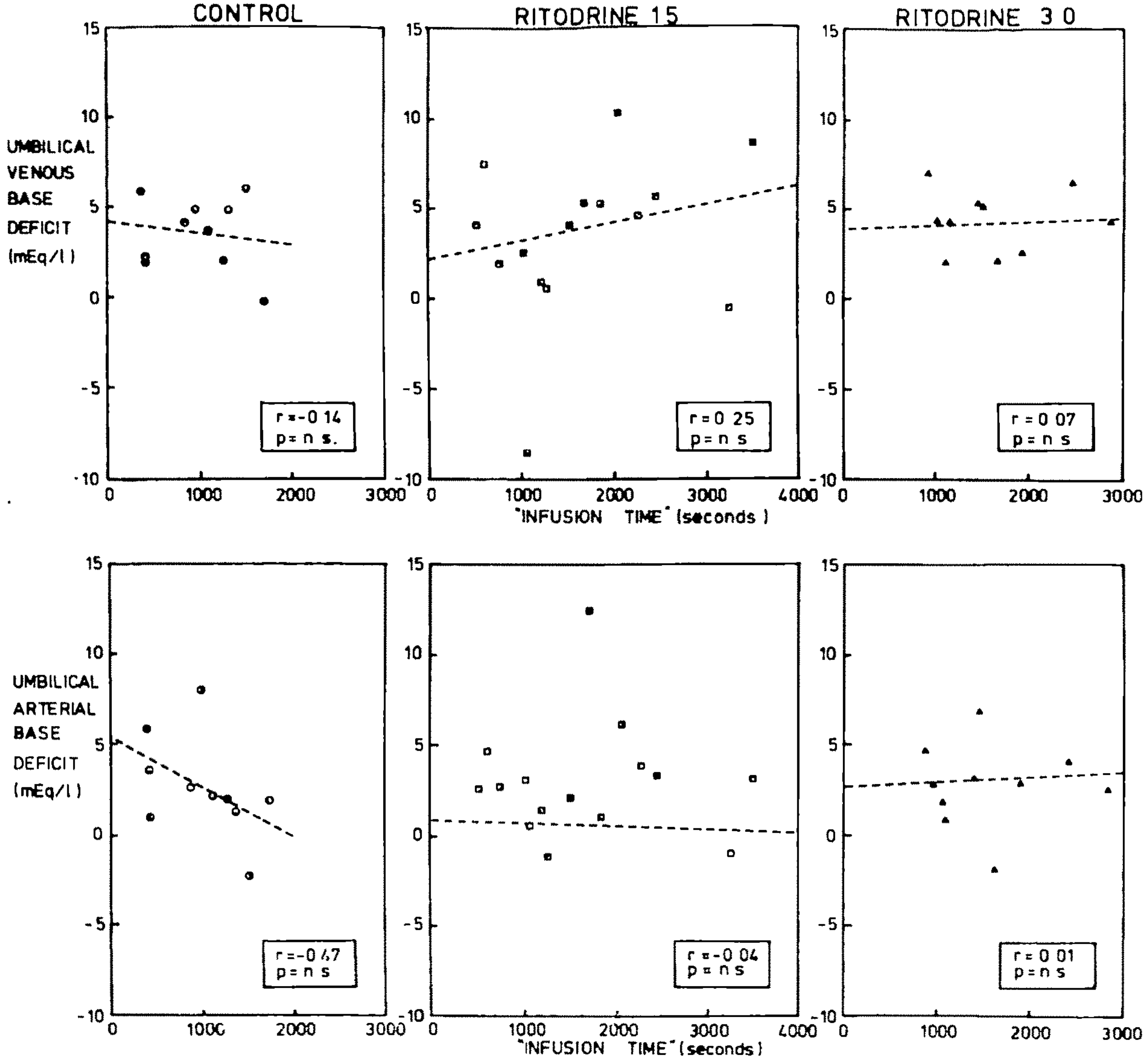
Control *vs.*
Ritodrine 3·0 μg./kg./minute
p<0·01

Control *vs.*
Ritodrine 1·5 μg./kg./minute
p=n.s.

Control *vs.*
Ritodrine 3·0 μg./kg./minute
p=n.s.

FIG. 4

Correlation of umbilical cord blood percentage oxygen saturation and "infusion time" (interval between start of infusion and delivery) in control and drug groups.



COMPARISON of REGRESSION LINES

Control *vs.*
Ritodrine 1·5 μg./kg./minute
p=n.s.

Control *vs.*
Ritodrine 3·0 μg./kg./minute
p=n.s.

Control *vs.*
Ritodrine 1·5 μg./kg./minute
p=n.s.

Control *vs.*
Ritodrine 3·0 μg./kg./minute
p=n.s.

FIG. 5

Correlation of umbilical cord blood base deficit at a haemoglobin level of 5 g./100 ml. and "infusion time" (interval between start of infusion and delivery) in control and drug groups.

TABLE II

*Umbilical cord blood acid-base and gas results, with cases divided into those delivering within 15 minutes (900 seconds) of starting the infusion, and those delivering after 15 minutes*

| | | Controls | | 1·5 $\mu$g./kg./minute Ritodrine | | 3·0 $\mu$g./kg./minute Ritodrine | |
|---|---|---|---|---|---|---|---|
| Infusion-delivery interval (seconds) | | <900 | >900 | <900 | >900 | <900 | >900 |
| Number of patients | | 4 | 6 | 3 | 12 | 1 | 9 |
| Umbilical venous pH | mean | 7·357 | 7·312 | 7·371 | 7·364 | 7·304 | 7·342 |
| | S.D. | 0·021 | 0·020 | 0·048 | 0·007 | — | 0·031 |
| | t | 3·40 (p<0·02) | | 0·21 (p=n.s.) | | — | |
| | F | 1·09 (p=n.s.) | | 0·84 (p=n.s.) | | — | |
| Umbilical arterial pH | mean | 7·272 | 7·263 | 7·313 | 7·283 | 7·252 | 7·299 |
| | S D. | 0·053 | 0·048 | 0·048 | 0·048 | — | 0·059 |
| | t | 0·33 (p=n.s.) | | 0·97 (p=n.s.) | | — | |
| | F | 2·47 (p=n.s.) | | 0·99 (p=n.s.) | | — | |
| Umbilical venous $pO_2$ (mm. Hg.) | mean | 28·8 | 22·7 | 29·1 | 30·1 | 29·0 | 27·0 |
| | S.D. | 6·3 | 6·3 | 4·5 | 5·2 | — | 5·0 |
| | t | 1·50 (p=n.s.) | | 0·30 (p=n.s.) | | — | |
| | F | 1·01 (p=n.s.) | | 0·75 (p=n.s.) | | — | |
| Umbilical arterial $pO_2$ (mm. Hg.) | mean | 14·6 | 14·0 | 16·2 | 16·8 | 17·3 | 16·0 |
| | S.D. | 4·0 | 3·6 | 1·3 | 4·4 | — | 4·6 |
| | t | 0·25 (p=n.s.) | | 0·23 (p=n.s.) | | — | |
| | F | 1·25 (p=n.s.) | | 0·09 (p=n.s.) | | — | |
| Umbilical venous $pCO_2$ (mm. Hg.) | mean | 38·5 | 46·1 | 34·2 | 38·0 | 37·8 | 39·4 |
| | S.D. | 2·6 | 4·8 | 7·8 | 9·1 | — | 4·9 |
| | t | 2·88 (p=0·02) | | 0·65 (p=n.s.) | | — | |
| | F | 0·29 (p=n.s.) | | 0·72 (p=n.s.) | | — | |
| Umbilical arterial $pCO_2$ (mm. Hg.) | mean | 51·1 | 57·2 | 45·5 | 53·1 | 50·4 | 48·6 |
| | S.D. | 6·7 | 5·2 | 8·4 | 8·0 | — | 5·2 |
| | t | 1·62 (p=n.s.) | | 1·47 (p=n.s) | | — | |
| | F | 1·63 (p=n.s.) | | 1·11 (p=n s.) | | — | |
| Umbilical venous base deficit (at Hb. 5 g./100 ml.) | mean | 3·5 | 3·6 | 5·0 | 3·4 | 6·9 | 3·8 |
| | S.D. | 1·8 | 2·3 | 2·3 | 4 9 | — | 1·5 |
| | t | 0·04 (p=n.s.) | | 0·53 (p=n.s.) | | — | |
| | F | 0·62 (p=n.s.) | | 0·23 (p=n.s.) | | — | |
| Umbilical arterial base deficit (at Hb. 5 g./100 ml.) | mean | 3·3 | 2·2 | 3·1 | 2·7 | 4·6 | 2·5 |
| | S.D. | 2·0 | 3·3 | 1·2 | 3·6 | — | 2·4 |
| | t | 0·58 (p=n.s.) | | 0·20 (p=n.s.) | | — | |
| | F | 0·37 (p=n.s.) | | 0·11 (p=n.s.) | | — | |
| Umbilical venous $O_2$ saturation (per cent) | mean | 65 0 | 45·0 | 69·7 | 69·2 | 64 0 | 59·8 |
| | S.D. | 8·1 | 20·3 | 4 7 | 8·5 | — | 15·4 |
| | t | 1·85 (p=0·1) | | 0·09 (p=n.s.) | | — | |
| | F | 0·15 (p=n.s.) | | 0·31 (p=n.s.) | | — | |
| Umbilical arterial $O_2$ saturation (per cent) | mean | 23·8 | 21·3 | 30·0 | 30·1 | 29 0 | 28·0 |
| | S.D. | 12·6 | 11·3 | 1·0 | 13·3 | — | 14·1 |
| | t | 0·32 (p=n.s.) | | 0·01 (p=n.s.) | | — | |
| | F | 1·24 (p=n.s.) | | 0·01 (p=n.s.) | | | |

TABLE III

*Statistical comparison of umbilical cord blood values in the patients delivering more than 15 minutes (900 seconds) after the start of the infusion*

| | | Controls *vs.* 1·5 µg./kg./minute Ritodrine | | Controls *vs.* 3·0 µg./kg./minute Ritodrine | |
|---|---|---|---|---|---|
| Number of patients | | 6 | 12 | 6 | 9 |
| Umbilical venous pH | mean | 7·312 | 7·364 | 7·312 | 7·342 |
| | S.D. | 0·020 | 0·007 | 0·020 | 0·031 |
| | t | 2·34 (p<0·05) | | 1·74 (p=n.s.) | |
| | F | 0·14 (p=n.s.) | | 0·25 (p=n.s.) | |
| Umbilical arterial pH | mean | 7·263 | 7·283 | 7·263 | 7·299 |
| | S.D. | 0·033 | 0·048 | 0·033 | 0·059 |
| | t | 0·93 (p=n.s.) | | 1·33 (p=n.s.) | |
| | F | 0·49 (p=n.s.) | | 0·32 (p=n.s.) | |
| Umbilical venous $pO_2$ (mm. Hg.) | mean | 22·7 | 30·1 | 22·7 | 27·0 |
| | S.D. | 6·3 | 5·2 | 6·3 | 5·0 |
| | t | 2·65 (p<0·02) | | 1·49 (p=n.s.) | |
| | F | 1·48 (p=n.s.) | | 1·63 (p=n.s.) | |
| Umbilical arterial $pO_2$ (mm. Hg.) | mean | 14·0 | 16·8 | 14·0 | 16·0 |
| | S.D. | 3·6 | 4·4 | 3·6 | 4·6 |
| | t | 1·34 (p=n.s.) | | 0·91 (p=n.s.) | |
| | F | 0·68 (p=n.s.) | | 0·63 (p=n.s.) | |
| Umbilical venous $pCO_2$ (mm. Hg.) | mean | 46·1 | 38·0 | 46·1 | 39·4 |
| | S.D. | 4·8 | 9·1 | 4·8 | 4·9 |
| | t | 2·01 (p=n.s.) | | 2·62 (p=0·02) | |
| | F | 0·27 (p=n.s.) | | 0·97 (p=n.s.) | |
| Umbilical arterial $pCO_2$ (mm. Hg.) | mean | 57·2 | 53·1 | 57·2 | 48·6 |
| | S.D. | 5·2 | 8·0 | 5·2 | 5·2 |
| | t | 1·14 (p=n.s.) | | 3·10 (p<0·01) | |
| | F | 0·43 (p=n.s.) | | 1·02 (p=n.s.) | |
| Umbilical venous base deficit (at Hb. 5 g./100 ml.) | mean | 3·6 | 3·4 | 3·6 | 3·4 |
| | S.D. | 2·3 | 4·9 | 2·3 | 4·9 |
| | t | 0·08 (p=n.s.) | | 0·27 (p=n.s.) | |
| | F | 0·22 (p=n.s.) | | 2·21 (p=n.s.) | |
| Umbilical arterial base deficit (at Hb. 5 g./100 ml.) | mean | 2·2 | 2·7 | 2·2 | 2·5 |
| | S.D. | 3·3 | 3·6 | 3·3 | 2·4 |
| | t | 0·29 (p=n.s.) | | 1·92 (p=n.s.) | |
| | F | 0·81 (p=n.s.) | | 1·92 (p=n.s.) | |
| Umbilical venous $O_2$ saturation (per cent) | mean | 45·0 | 69·2 | 45·0 | 59·8 |
| | S.D. | 20·3 | 8·5 | 20·3 | 15·4 |
| | t | 3·61 (p<0·01) | | 1·61 (p=n.s.) | |
| | F | 5·71 (p<0·01) | | 1·73 (p=n.s.) | |
| Umbilical arterial $O_2$ saturation (per cent) | mean | 21·3 | 30·1 | 21·3 | 28·0 |
| | S.D. | 11·3 | 13·3 | 11·3 | 14·1 |
| | t | 1·37 (p=n.s.) | | 0·97 (p=n.s.) | |
| | F | 0·72 (p=n.s.) | | 0·65 (p=n.s.) | |

values of the treated patients were higher than those of the control patients, and $pCO_2$ values were lower. These differences were significant for umbilical venous pH, $pO_2$ and oxygen saturation in the low dose Ritodrine group, and for umbilical arterial and venous $pCO_2$ in the high dose Ritodrine group.

## DISCUSSION

The three groups of patients were found to be comparable in all obstetric parameters examined. Maternal pH was slightly lower in the high dose Ritodrine group, but this is not likely to greatly affect fetal condition (Lumley *et al.*, 1970).

The control group showed the progressive development of a respiratory acidosis throughout the second stage of labour, these changes being most marked in the umbilical venous blood. This confirms previous work (Humphrey *et al.*, 1973; Wood *et al.*, 1973*a* and *b*). The patients given Ritodrine did not show this progressive deterioration—in fact, $pO_2$ and oxygen saturation improved during the infusion. In general, the improvements were more marked in the group of patients receiving the larger dose of Ritodrine. though the differences between the two treatment groups were not statistically significant.

Table II shows the magnitude of the changes which occur in untreated patients, and which are avoided by the use of Ritodrine. In the control group there was a difference of 0·045 pH units between those delivered before and after 15 minutes. The difference in umbilical venous $pO_2$ was 6·1 mm. Hg, in $pCO_2$ was 7·6 mm. Hg., and in oxygen saturation was 20 per cent.

A number of hypotheses can be put forward to explain the improvement in fetal condition. Firstly, being a sympatho-mimetic agent, Ritodrine may directly increase fetal cardiac output, and hence may increase fetal blood flow through the placenta. This may allow for greater gas exchange. Secondly, placental separation may occur in the second stage of labour, prior to the delivery of the fetus. Ritodrine, by decreasing uterine tone, could prevent such separation. This type of effect would be variable and the wider scatter of results in the Ritodrine treated group would be consistent with this explanation. Thirdly, the strong contractions of the second stage of labour diminish maternal placental blood flow (Borell *et al.*, 1965; Cosmi and Condorelli, 1973; Renaud *et al.*, 1973*b*). Ritodrine, by decreasing the strength of the uterine contractions may minimize this effect. This theory would be consistent with the amelioration of the time related pH changes previously demonstrated with the avoidance of the supine delivery position (Humphrey *et al.*, 1974). And lastly, maternal blood flow through the placenta may be improved by a direct vasodilator effect of Ritodrine on uterine blood vessels. It is not possible to decide, from this study, whether one or more of the above hypotheses is valid.

There is no evidence that Ritodrine has serious side effects for the mother or for the fetus (Renaud *et al.*, 1973*a*; Caldeyro-Barcia *et al.*, 1969; Sivasambo, 1973; Thiery *et al.*, 1973; Renaud *et al.*, 1973*c*; Decelle and Vokaer, 1973). In particular, postpartum haemorrhage, which might be expected because Ritodrine is a uterine relaxant, did not occur in the present study. Furthermore, the incidence of postpartum haemorrhage was not increased in patients given Ritodrine for treatment of premature labour and acute fetal distress.

We suggest that an intravenous infusion of Ritodrine in the normal second stage of labour, combined with low forceps delivery to prevent prolongation of the second stage resulting from Ritodrine (Niswander and Gordon, 1973), will result in fetuses in better condition. A larger controlled clinical trial would test this hypothesis and also determine whether the expected improvement in pH and oxygen status is clinically important for the fetus and newborn. Where the fetus is at risk the use of Ritodrine and forceps may be particularly appropriate.

## CONCLUSIONS

A respiratory acidosis normally occurs during the second stage of labour. This potentially detrimental effect may be abolished by the intravenous infusion of Ritodrine throughout the second stage. Further studies are needed to be able to understand the physiological basis of this effect and the place of Ritodrine in obstetric management.

## ACKNOWLEDGEMENTS

The help of the labour ward nursing staff of the Queen Victoria Memorial Hospital was invaluable. We are indebted to Professor W. A. W. Walters for his helpful criticism, Philips-Duphar Pty. Ltd., for supplies of Ritodrine and placebo, and to the Felton Bequest for financial support.

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# DILATATION OF THE PAROUS NON-PREGNANT CERVIX

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## Summary

*In vitro* cervical dilatations were carried out on 33 uteri removed by hysterectomy for conditions not involving the cervix from parous women in their reproductive span of life. A specially designed force measuring dilator was used for all the tests. The analysis of the results involved the application of a thick-walled cylindrical stress determination technique. Using this approach it was possible to calculate quasi-elastic moduli for different degrees of dilatation and obtain theoretical distributions of radial and circumferential stress across the cervical wall. These results indicated a yielding of the cervical tissue at about 9 to 11 mm. dilatation in a significant number of cases.

IN 1968 the Japanese and the Hungarians first noted an increase in mid-trimester abortion and premature labour in gestations following vaginal termination of pregnancies (Potts, 1970). Independent surveys by Wright *et al.* (1972) and Liu *et al.* (1972) reported a four- to tenfold increase in mid-trimester miscarriages subsequent to vaginal abortions. The incidence of obvious cervical laceration lies between 3 to 5 per cent (Stallworthy *et al.*, 1971; Liu and Hudson, 1974*a* and *b*) and, as expected, increased with the need for greater dilatation of the cervix required in terminations over 14 weeks gestation (Liu and Hudson, 1974). It is the purpose of this study to assess how the non-pregnant parous cervix tears and what is the limit of dilatation below which damage would be minimal.

## METHODS

After some initial recordings from *in vivo* prehysterectomy cervices, all the tests on which this paper is based were done *in vitro* on uteri removed for conditions not involving the cervix. The operation was carried out at mid-cycle to standardize hormonal effects on the cervix (Palmer and Liesse, 1946; Asplund, 1952). To avoid the possibility of primary cervical incompetence (Brooks, 1963) all specimens were from multiparous women between 30 and 50 years of age with a history of normal deliveries.

Logically the best approach was to subject the

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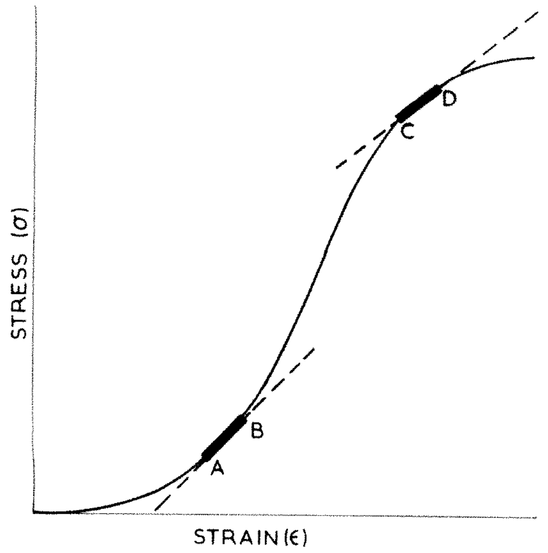
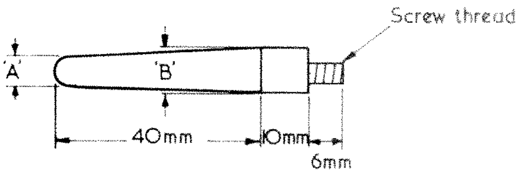


FIG. 1

Typical stress/strain relationship for visco-elastic material with straight line segments of non-linear stress/strain relationship.



RANGE OF INTERCHANGEABLE DILATORS

| A | B | A | B |
|---|---|---|---|
| 2 mm | 4 mm | 3 mm | 5 mm |
| 4 mm | 6 mm | 5 mm | 7 mm |
| 6 mm | 8 mm | 7 mm | 9 mm |
| 8 mm | 10 mm | 9 mm | 11 mm |
| 10 mm | 12 mm | 11 mm | 13 mm |
| 12 mm | 14 mm | 13 mm | 15 mm |

FIG. 2

Details of dilator head sizes.

intact cervix to a uniform axial force (as when graduated dilators were passed) and the response in the form of a stress/strain relationship could be assessed. Any large increment in strain without a proportional increase in stress would indicate excessive yielding or possibly rupture of collagen fibres. The cervix, being essentially collagenous connective tissue (Danforth, 1947), responds to stress according to a curve (Figure 1) characteristic of visco-elastic tissue (Vhdik and Ekholm, 1968), i.e. a slow rise of stress in response to strain and then a sharp rise of stress to strain.

Direct application of a straight line equation to the curve of visco-elastic behaviour is not a reasonable approximation. However, small segments of the visco-elastic curve corresponding to each stage of cervical dilatation can be assumed straight as illustrated by A–B and C–D in Figure 1, and a modulus can be obtained for each dilatation. This quasi-elastic modulus, which represents the gradient of a stress/strain relationship, will increase with each stage of dilatation. Failure to increase indicates a stretching of the material.

A series of detachable dilator heads which increased 2 mm. in diameter along a slope of 1 in 40 were made (Fig. 2). These heads were screwed onto a pistol-grip handle containing a force measuring transducer and the forces required to dilate the cervix were recorded on an XY chart (Fig. 3).

The cervix was sewn onto a ring holder (Fig. 4) to allow uniform circumferential countertraction and to attempt to simulate the range of movements obtaining *in vivo*. Because the response of visco-elastic materials to deformation is time dependent, i.e. a fast deformation requires more force than the same extent of deformation achieved at a slower rate, we have standardized the passage of each dilatation to approximately five seconds.

The shape of the cervix lends itself readily to consideration as a thick-walled cylinder. With the small series used and to allow mathematical representation, a number of assumptions were made.

The cervix was assumed to be elastic, isotropic, incompressible, homogeneous and cylindrical with a diameter of 25 mm. and length of 25 mm. The assumption of incompressibility implied that the value of Poisson's Ratio (which is a material constant) was 0·5.

Having taken into consideration the frictional resistance and the unknown recovery rate of

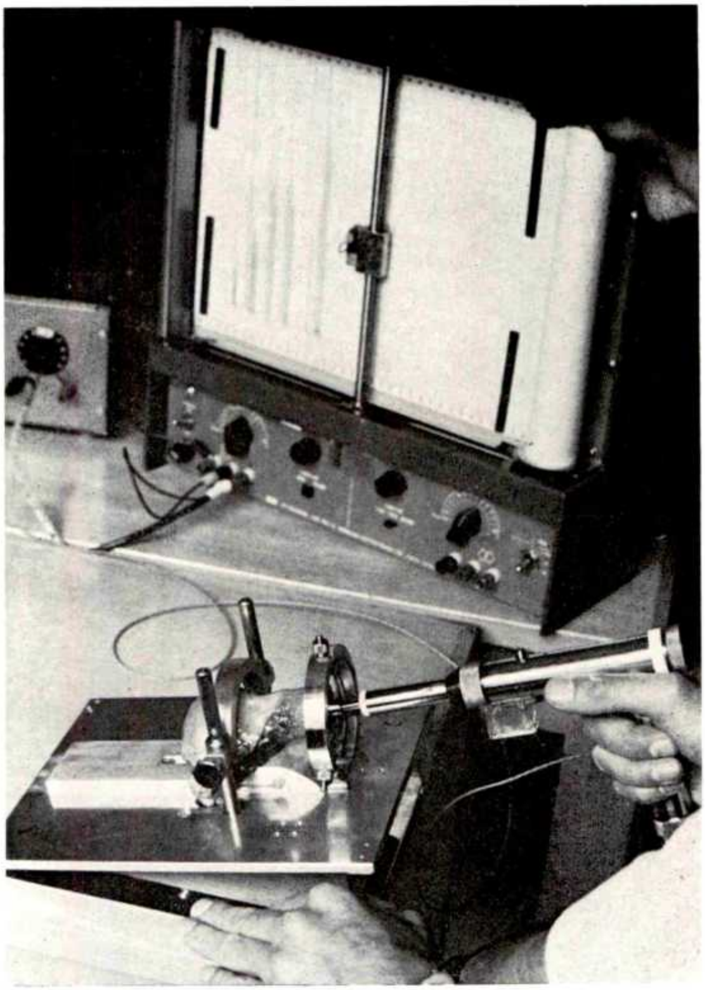


FIG. 3
*In vitro* dilatation in progress showing force recording chart.

cervical tissue after stretching (the magnitude of these forces falls within the range of test error and was not considered significant), the recorded axial force was resolved into the actual radial force acting on the cervix. This involved calculation of the active area of the propagating dilator (which is an annulus) and derivation of the force acting perpendicular to the cervical wall when the angle formed by the slope of 1 in 40 was considered. Employing Lamé's equations for thick walled cylinders and substituting the calculated pressure, a quasi-elastic modulus (quasi—because of the assumptions used) was obtained for each dilatation in every experiment.

## RESULTS

In all, 33 sets of experimental readings were accepted for analysis. Values of radial stress ($\sigma_R$) and circumferential stress ($\sigma_\theta$) were calculated for a range of increasing dilatations and at increments of radii across the cervical wall. Using these results, it was possible to plot the distribution of these stresses across the thickness of the cervical wall. Typical examples of such distribution are shown in Figures 5 and 6.

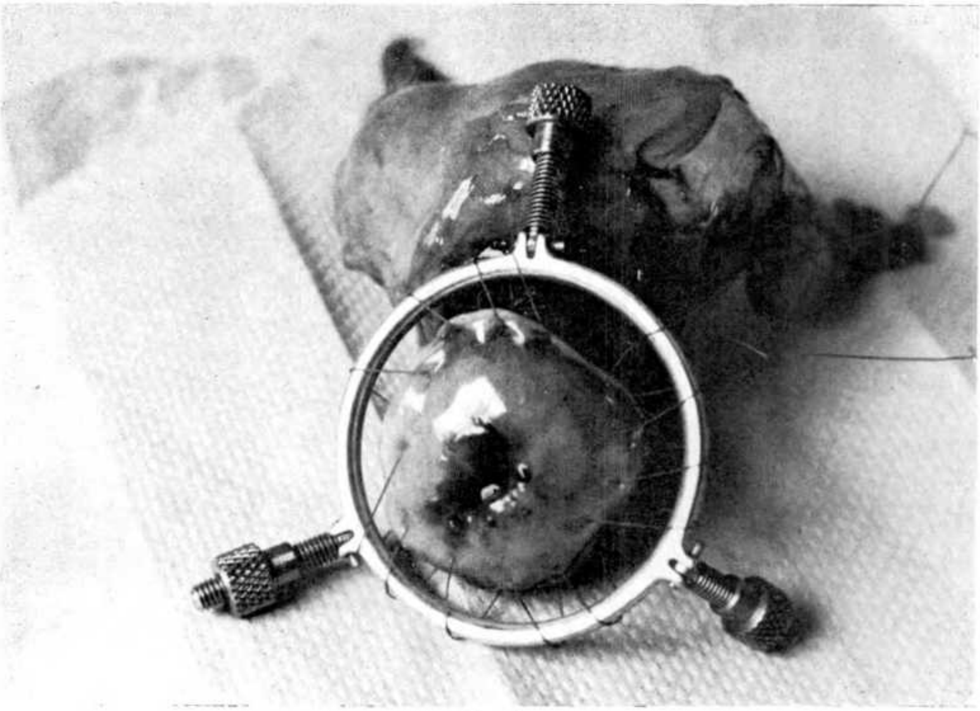


FIG. 4
Attachment of cervix to counter-traction ring.

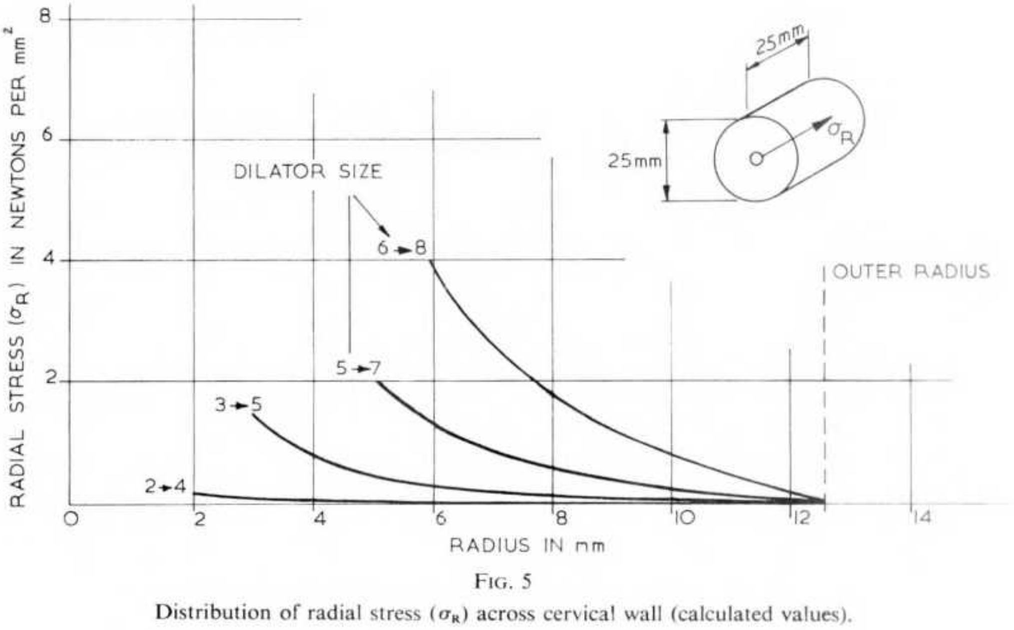


FIG. 5
Distribution of radial stress ($\sigma_R$) across cervical wall (calculated values).

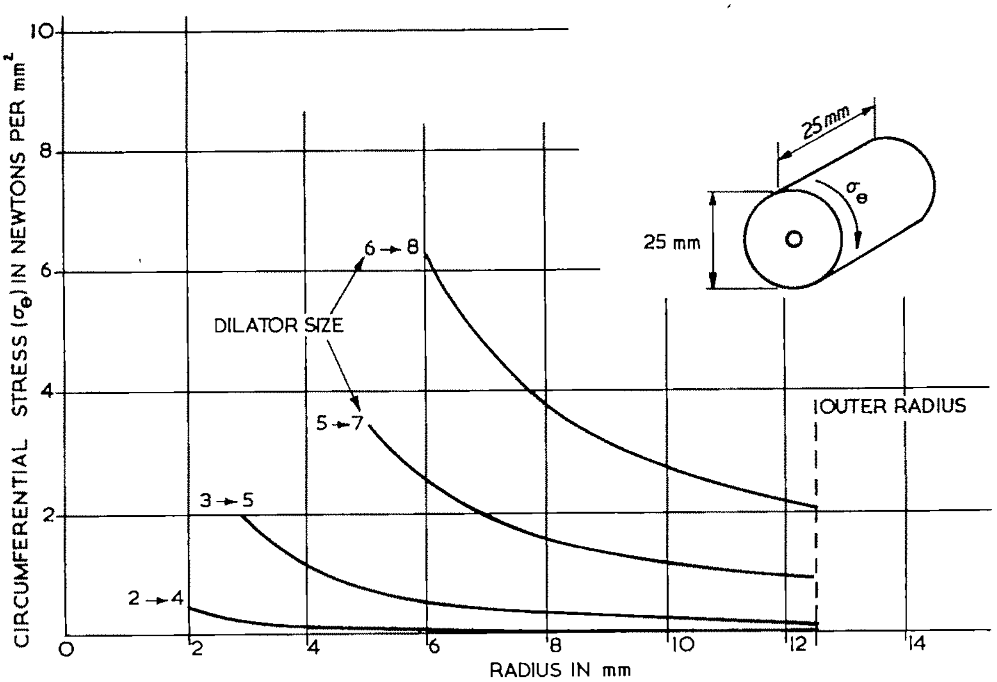


FIG. 6
Distribution of circumferential stress ($\sigma_\theta$) across cervical wall (calculated values).

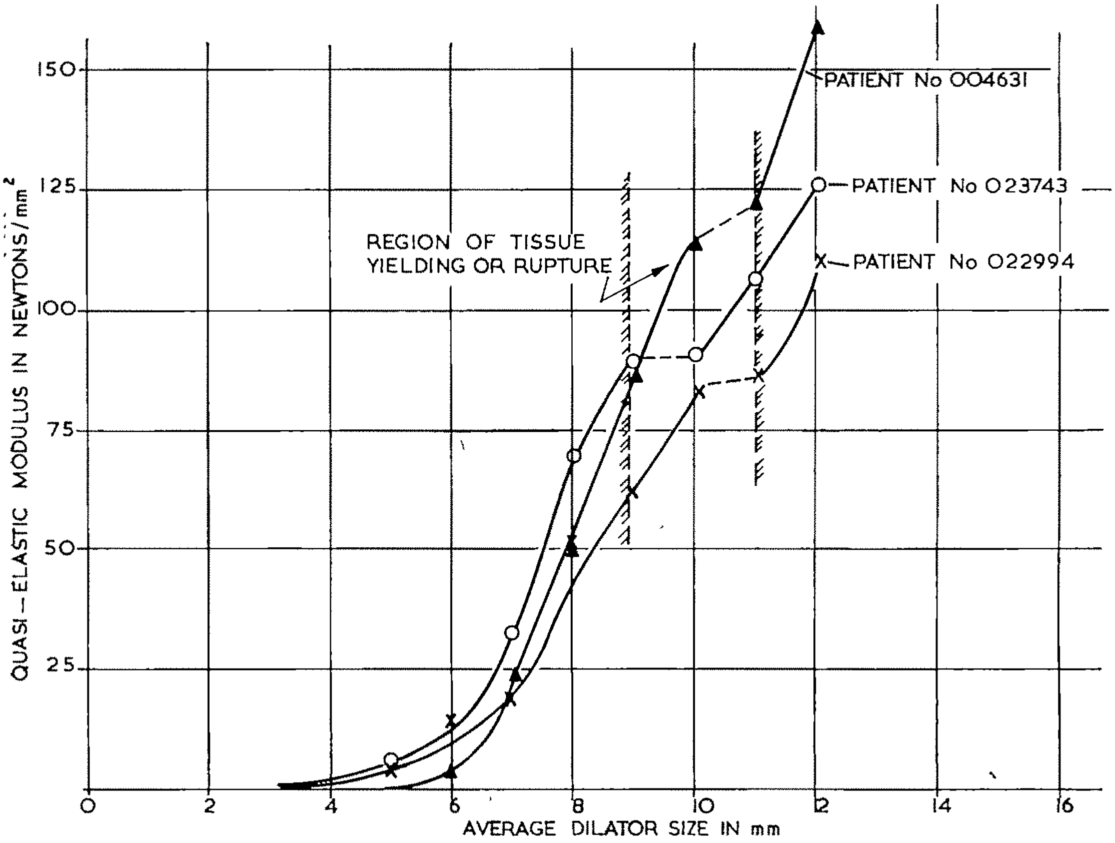


FIG. 7
Variation of quasi-elastic modulus with dilator size.

From these graphs it can be seen that both radial and circumferential stress decay rapidly and non-linearly towards the outer boundary of the cervical wall. In the case of radial stress the limiting value is zero (which it must be from consideration of stress equilibrium) and for circumferential stress the corresponding value is approximately 25 to 30 per cent of the circumferential stress at the inner radius.

There was no apparent initial stretching of the peripheral circumference to accommodate the gradual internal dilatation until a stage was reached when dilatation caused macroscopic tissue rupture. In 95 per cent of the tests, a radial tear, commencing from the inside, was observed at dilatations of 13 to 14 mm. diameter.

In almost 50 per cent of the specimens tested, a marked change of slope of the graph of calculated quasi-elastic modulus against dilatation was observed at dilatations of approximately 9 to 11 mm. diameter as shown typically for three cases in Figure 6. This phenomenon would suggest that a yielding or initial rupture of tissue, not necessarily visible to the naked eye, was obtaining at this magnitude of dilatation. In the remainder of the tests, this slope change was observed at higher values of dilatation.

## DISCUSSION

The validity of the assumptions used for the theoretical analysis can only be assessed as acceptable if the calculated results correlate with clinical findings. It is common experience that during cervical dilatation of the non-pregnant parous patient, within her reproductive life span, a reduction in the resistance to dilatation is usually noticed when the 8 mm. to 11 mm. diameter dilators are used.

Almost half of the calculated results correlated with clinical findings. The remainder showed similar behaviour at larger values of dilatation. The calculated distributions of radial and circumferential stress correlated with the observed tearing from inside outwards, as the cervix split when dilatation was excessive. If cervical compliance is defined as the accommodating ability of the cervix to a mechanical dilating force, the above results indicate that, in a parous non-pregnant cervix, a suggested limiting value of this compliance, if tissue damage is to be avoided, is 10 mm. diameter. Although the effects of gestational hormones would undoubtedly extend this limiting compliance, it would be best not to assume that this is the case.

## ACKNOWLEDGEMENTS

We are grateful for the support of Mr. Brian Measday, the theatre staff at the New Sussex Hospital and colleagues from the Graduate Division of Biomedical Engineering at the University of Sussex. This project was supported financially by the South East Thames Regional Health Authority through a Research Fellowship to David T. Y. Liu.

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*British Journal of Obstetrics and Gynaecology*
March 1975 Vol. 82 pp. 252–254

# PAPERS PRESENTED AT A MEETING OF THE BLAIR BELL RESEARCH SOCIETY AT THE ROYAL COLLEGE OF OBSTETRICIANS AND GYNAECOLOGISTS ON 7TH JANUARY 1975

## PLASMA OESTROGEN LEVELS FOLLOWING INTRAVENOUS INJECTION OF DEHYDROEPIANDROSTERONE SULPHATE IN THE THIRD TRIMESTER OF PREGNANCY

BY
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A 100 mg. dose of dehydroepiandrosterone sulphate (DHAS) was given intravenously to 70 women in the third trimester of pregnancy with the aim of developing a test of placental "reserve". Unconjugated oestradiol (E2) and oestriol (E3) were measured in plasma over at least a two-hour period in every test. In some patients unconjugated oestrone (E1), total E2 and total E3 were also measured. Radioimmunoassays of the oestrogens were carried out using an antibody raised in sheep to oestradiol-17β-hemisuccinate-bovine serum albumin. Separation of the oestrogens was achieved by chromatography on columns of Sephadex LH-20. Column recovery for E2 was 82·1 per cent (SD = 4·12; n = 29), for E3 86·0 per cent (SD = 4·75; n = 29) and for E1 74·7 per cent (SD = 5·71; n = 30).

In all 21 normal pregnancies there was a dramatic rise in unconjugated E2 reaching a peak of 60 to 180 ng./ml. within 30 to 90 minutes and returning to baseline within 6 to 18 hours. The percentage increase in E2 at 60 minutes varied between 174 and 478 per cent (mean 304 per cent; SD ±85·6). The height of the peak was not closely correlated with basal levels and there was often a marked fluctuation of plasma E2 levels between 30 and 120 minutes. In some patients plasma E3 tended to rise slightly after one to three hours but this was extremely variable, rarely exceeded a 50 per cent increase and sometimes even fell. Plasma E1 also showed variable changes, sometimes rising markedly and sometimes showing virtually no rise.

In almost all the 49 tests on patients with pregnancy complications there was also a dramatic rise in plasma E2 identical in type to that seen in normal pregnancies, demonstrating a remarkable reserve of steroidogenic potential even in compromised pregnancies. This occurred even in patients with severe pre-eclampsia who had intrauterine growth retardation, low basal plasma E2 and E3 levels and low falling urinary oestrogen excretion. Several pre-eclamptic patients had very high peak levels of E2 which did not correlate with clinical state. Diabetics and twin pregnancies tended to have high basal values and high E2 peaks, one binovular twin pregnancy reaching an E2 peak of 317·6 ng./ml. at one hour. One patient who had experienced intrauterine fetal death 11 days previously (at 36 weeks) had very low basal E2 levels, but a response well within the normal range. A patient who was thought to have a placental sulphatase deficiency showed no response whatsoever, and help in diagnosis of this condition appears to be the only value of the test as described here.

# CONVERSION OF DEHYDROEPIANDROSTERONE TO OESTROGEN—A DYNAMIC PLACENTAL FUNCTION TEST

BY
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WE have explored the conversion of dehydroepiandrosterone sulphate (DHAS) to oestrogens by the placenta. In this respect two pathways are possible. The free dehydroepiandrosterone (DHA) might first be converted to androstenedione and then to oestradiol via testosterone. By this route oestrone would also be formed directly from androstenedione and might give rise to further oestradiol by conversion in the mother. The alternative is the direct conversion of DHAS to testosterone and thence to oestradiol. We studied the response to 50 mg. i.v. DHAS in 12 patients with normal singleton pregnancies of 36 or more weeks gestation. Plasma oestradiol and testosterone levels were estimated using radioimmunoassay techniques.

The plasma oestradiol started rising within five minutes of administering intravenous DHAS. From a baseline level of 25 ng./ml. it rose to a peak of 102 ng./ml. and thereafter remained at that level for 3½ hours; at the end of that period it started declining, gradually returning to baseline level by 18 hours after injection. The response in testosterone levels was more dramatic, the rise being noted within two minutes of injection. From a baseline level of 1·5 ng./ml. it rose to 5·2 ng./ml. and very quickly the level came down to a level slightly above baseline. The testosterone peak lasted only 15 minutes. From these observations we feel that DHAS is converted to oestradiol via the testosterone pathway.

Plasma oestriol levels were studied and there was no significant change in these levels. This is probably due to substrate competition, DHAS and fetal 16-OHDHAS competing for the same placental enzymes.

The response to 50 mg. of DHA administered orally was studied in order to determine how far placental sulphatase activity was rate limiting in these conversions. Following oral administration of 50 mg. of DHA, the plasma oestradiol rose to 232 per cent above the baseline. Peak levels were achieved 90 minutes after the administration of DHA. A positive correlation was obtained between placental weight and plasma oestradiol levels 60 minutes after injection.

From all the data obtained we feel that conversion of DHAS to oestradiol represents only the placental function. The conversion pathway is probably via testosterone. The future of this test lies in examining separately the enzyme systems involved by using different substrates, e.g. DHA, androstenedione or testosterone.

# FETAL LUNG SURFACTANT—CHANGES IN COMPOSITION DURING LATE PREGNANCY AND THE POSSIBLE ROLE OF CORTISOL

BY
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AND
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THE use of the amniotic fluid lecithin/sphingomyelin (L/S) ratio in the study of fetal lung maturity is now well established in clinical practice. However, the techniques used are in all cases very simple and yield no information on the composition of the physiologically active "lecithin" fraction. This in turn hinders studies on the control of "lecithin" formation in the fetal lung.

A method was therefore developed for the gas chromatographic estimation of the methyl esters of the fatty acids derived from the total "lecithin" fraction prepared from amniotic fluid samples by standard techniques. Combined hydrolysis and esterification of the various fractions was carried out by refluxing with a sulphuric acid/methanol mixture. Gas chromatography was carried out using a 10 per cent EGSS-X column at 180 °C. A number of fatty acids was detected and quantified. The major components were correlated with the clinical history and L/S ratio.

In common with other workers it was clearly shown that palmitic acid predominated after 35 weeks of pregnancy but at least three other fatty acids were present in significant amounts. Before 35 weeks a much more variable pattern was seen. Results from various pathological pregnancies support the theory that maturation of dipalmityl lecithin synthesis is a vital link in fetal lung maturation. In particular, palmitic acid represented a significantly lower percentage ($P = 0 \cdot 001$) of the total fatty acid content in cases where the L/S ratio was less than $2 \cdot 0$. Preliminary evidence supports the clinical concept of the importance of corticosteroids in fetal lung maturation.

Physiological studies on specific corticosteroid binding macromolecules in the fetal lung of the rat and rabbit have been commenced using sucrose gradient analysis. Binding of $^3$H-cortisol was detected and it was shown that unlabelled cortisol and betamethasone abolished this binding. It is hoped that further animal studies will give useful information on the detailed mechanisms of corticosteroid "control" of synthesis of fetal lung surfactant.

## PROLONGED BLADDER DISTENSION AS A TREATMENT OF URGENCY AND URGE INCONTINENCE OF URINE

BY

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PATIENTS with urgency and urge incontinence were diagnosed as having detrusor instability after a careful history, a full clinical examination and bladder pressure studies had been carried out. Patients in whom specific treatable causes were discovered for their bladder instability were excluded.

Twenty-five patients were treated by prolonged bladder distension under epidural anaesthesia. Cystoscopy was carried out followed by transurethral passage into the bladder of an 18 French gauge Foley catheter with a condom securely tied to its end just proximal to the balloon, The bladder was distended hydrostatically by filling the condom to a pressure equal to the patient's systolic blood pressure for a total period of two hours. The bladder capacity was measured at cystoscopy, at 30 minute intervals during distension and, finally, at the end of distension after which the catheter was removed.

Thirteen of the 25 patients become symptom-free after prolonged bladder distension, 10 patients were improved, whilst in the remaining two patients symptoms remained unchanged. Post-operative cystometry became normal in 15 patients. The period of follow-up varied from 9 to 16 months after bladder distension. Complications were few, one patient had backache at the site of the epidural for six weeks and one patient had transient retention of urine immediately postoperatively.

The mechanisms by which prolonged bladder distension reverses detrusor instability is not fully understood but these early results would seem sufficiently impressive to justify a more extensive trial although the pressure and the duration of distension may require modification. It is not known whether the good results will be sustained or whether symptoms may return.

*British Journal of Obstetrics and Gynaecology*
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# BOOK REVIEWS

**Ultrasound in the Diagnosis of Abdominal Conditions.** By E. Barnett and P. Morley. Pp. x+138, with 118 figs. and 1 table. Butterworths, London, 1974. Price: £4.50.

Over the years the authors have been recognized as pioneers in the use of ultrasound for the diagnosis of abdominal conditions and the quality of their work has always been of the highest order. They now share some of their experience with us in this well-produced book. The profusion of scans illustrated brings the book close to being an ultrasonic atlas of the abdomen. About half of the material is of direct relevance to obstetricians wishing to make ultrasonic diagnosis one of their skills, the chapters on Obstetrics, Gynaecology and Physical Principles being most important. The chapters on general abdominal scanning also contain much of interest. Probably the major value of the work is that it shows the very large range of diagnoses possible and encourages the ultrasonographer to extend the often rather limited range of conditions he is prepared to investigate. It would be helpful to know if any of the conditions illustrated cannot be diagnosed prospectively under clinical conditions. The few factual errors which have gone unnoticed are happily of a trivial nature; for instance, the reflection at a solid/gas interface is not 90 per cent, but nearer 99·9 per cent. The book inevitably suffers, as does any text relating to a rapidly developing field, in that the clinical and instrumental techniques described are already changing by the time of publication. Thus differential diagnoses achieved by changing the probe frequency can now be achieved with a single probe on a high sensitivity instrument with versatile swept gain facilities. Also the most recent equipment has a grey scale display devoted to a small amplitude echoes and this type of display is causing a marked change in the appearance of scans. The text makes clear that many conditions cannot be successfully diagnosed with ultrasound, but it is not strongly emphasized that in inexperienced hands ultrasound scans are very susceptible to artifact. The interpretation of many scans illustrated depends upon complete freedom from unrecognized artifact and in the hands of the optimistic novice unfortunate misdiagnoses could result. In spite of this, the book is good value and makes a very positive contribution to the rather limited literature on the subject.

*R. Blackwell*

**Histopathologie Placentaire.** By E. Philippe. Pp. 247, with 259 figs. Masson and Cie, Paris, 1974. Price: 150 F.

This book will be invaluable to anyone working in the field of reproduction and interested in placental function and its disturbances. The first of the twelve chapters contains a very short survey of the reproductive process starting with ovulation and finishing with the delivery of the placenta. The next two chapters, more practical in nature, deal with techniques of examining placental tissue and the products of abortion, both macroscopically and microscopically. They also contain a most useful alphabetic illustrated lexicon of the commoner placental lesions. Then follow two chapters on the umbilical cord, the membranes and the amniotic fluid with their abnormalities. The remaining chapters are devoted to the changes that occur in most of the recognized pathological states, ranging from chorio-angioma and arterial thrombosis to diabetic and syphilitic placentae. It ends with a chapter on the placentae of ectopic pregnancies and a final one on moles entitled "The Trophoblastic Disease". The book has many illustrations but their quality is for the most part well below the high standard that Masson, the publishers, can obtain; they nevertheless convey the author's messages. The most notable feature of the book is the wide and deeply researched bibliography. One quarter of all the pages are taken up with some 2000 references closely printed in a smaller type than the rest of the book; few date from before 1960 and many are as recent as 1972. Over half the references are in English and for this reason I would recommend that the book be acquired by every library of gynaecology and pathology. Apart from this, the book is worth having for the splendid chapter on the substances that pass through the placenta and for the list of teratogenic drugs and viruses and their effects.

*E. Philipp*

**Transfusion of Blood by Freezing.** By S. Sumida, M.D. Pp. xiv+92, with 117 figs. and 11 tables and 15 plates in colour. Georg Thieme Verlag, Germany, 1974. Price: DM 58.

This interesting monograph is written for specialists in blood transfusion. The first part described cryomicroscopic observations of the changes which take place when blood is frozen in both aqueous media and cryoprotective agents. There are many excellent photomicrographs. Unfortunately, the English text of this part of the book is sometimes a little difficult to follow but the style changes when the author describes the practical problems of blood banking using frozen blood. Dr. Sumida uses a modification of Huggin's low-freeze, high-glycerol technique. The blood is stored at —80 °C. in polyvinylchloride bags. No indication is given of a limit to the length of time for which the blood is stored but in Britain it is usually used within two years of donation. Careful assessment has been made of haemoglobin in recovery rate, 24-hour *in vivo* survival, and intracellular ATP and potassium concentrations. Slightly better results are obtained using the low-glycerol rapid-freeze technique which we use in Britain but the liquid nitrogen containers, which are necessary to store the blood at —196 °C., are too bulky when, as in Japan, large volumes of frozen blood are used. The main advantage of using frozen blood is that, during the deglycerolization process, most of the white cells and platelets are removed and it is thus ideal for transfusion into patients with white cell and platelet antibodies. The second main advantage of storing blood in the frozen state is to iron out seasonal variations in blood donations in order to prevent blood shortage during holiday periods. Dr. Sumida fails to stress that the reclaiming process is an open one and that the blood must therefore be used within 12 hours. This limits the use of frozen blood in Britain where many transfusion centres are very far from the limits of the area which they supply. The cost of the process was not mentioned but in terms of both hardware (large capacity deep freezers) and software (complex plastic bags and transfer packs) it must be very high.

*H. Dodsworth*

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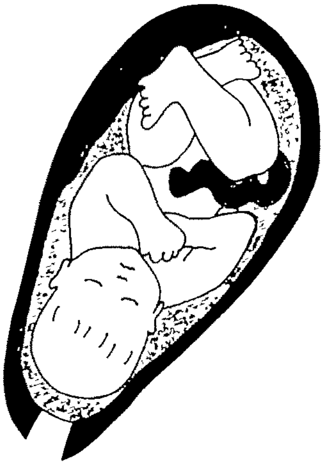

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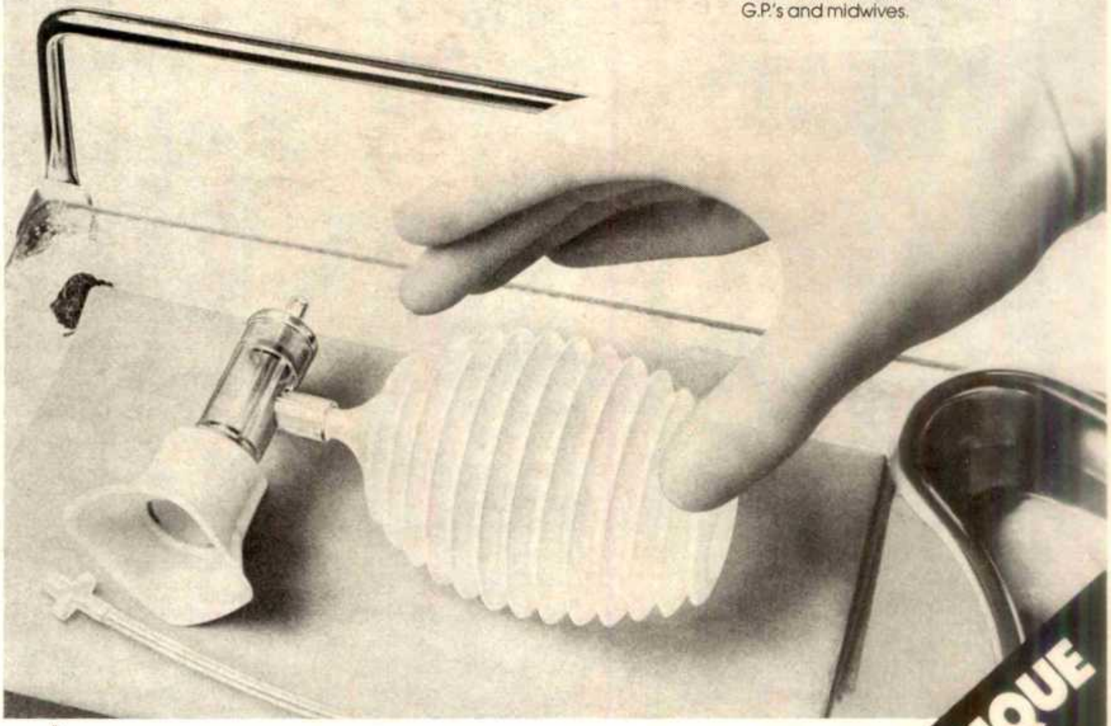

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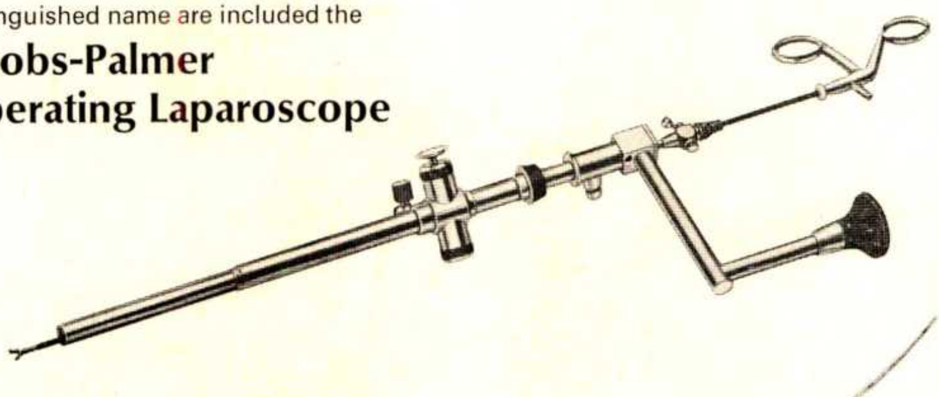

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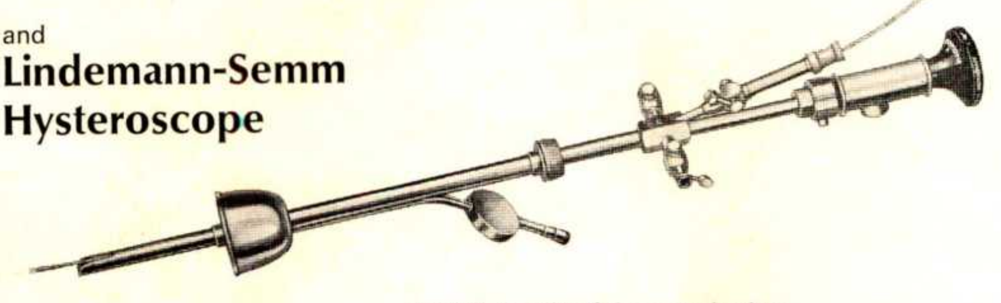

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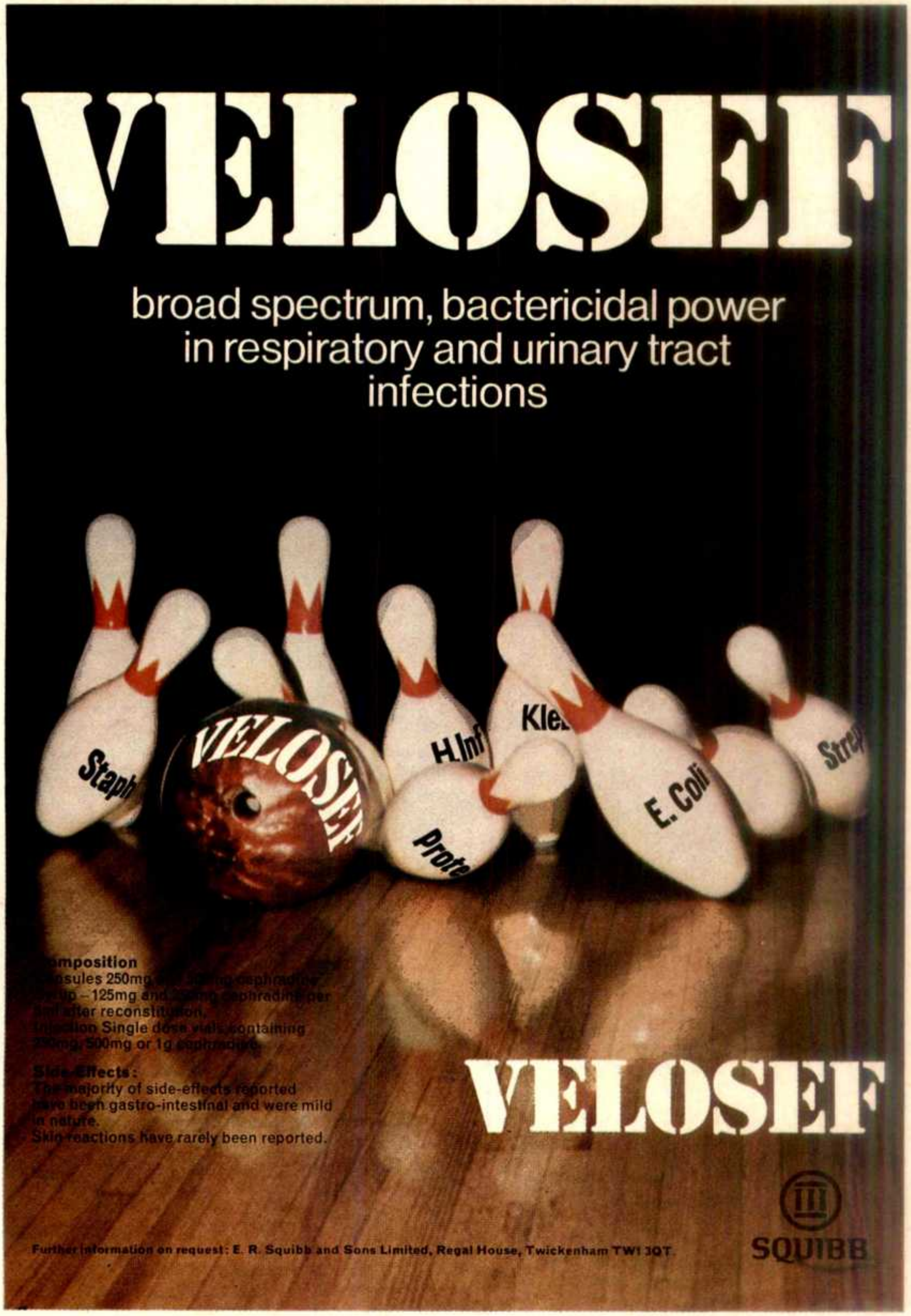

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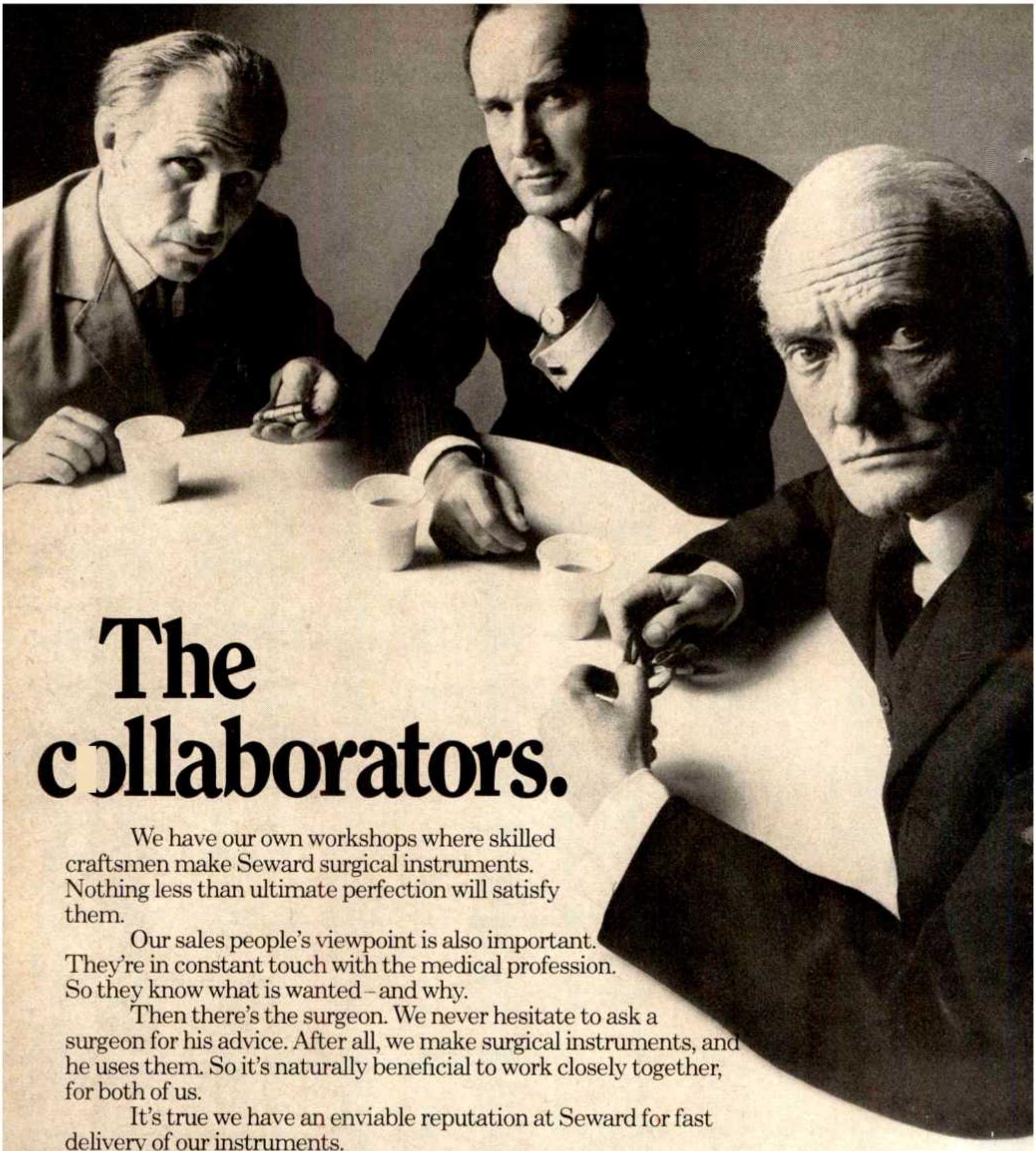

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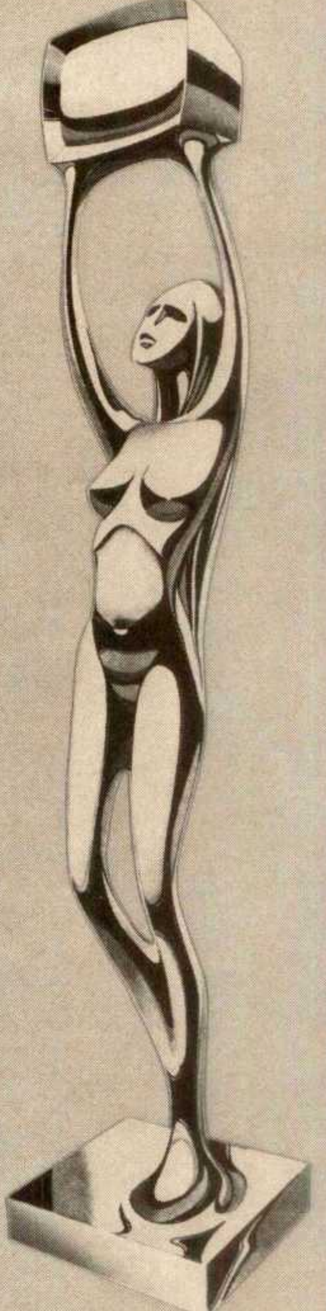

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1 **World Med., 8,** (19), 73, 1973
2 **Gen. Practit.,** p. 19, 13 July, 1973
3 **Practitioner, 212,** 115, 1974
4 **Br.Med.J., iv,** 146, 1971
5 **Ibid., i,** 207, 1971

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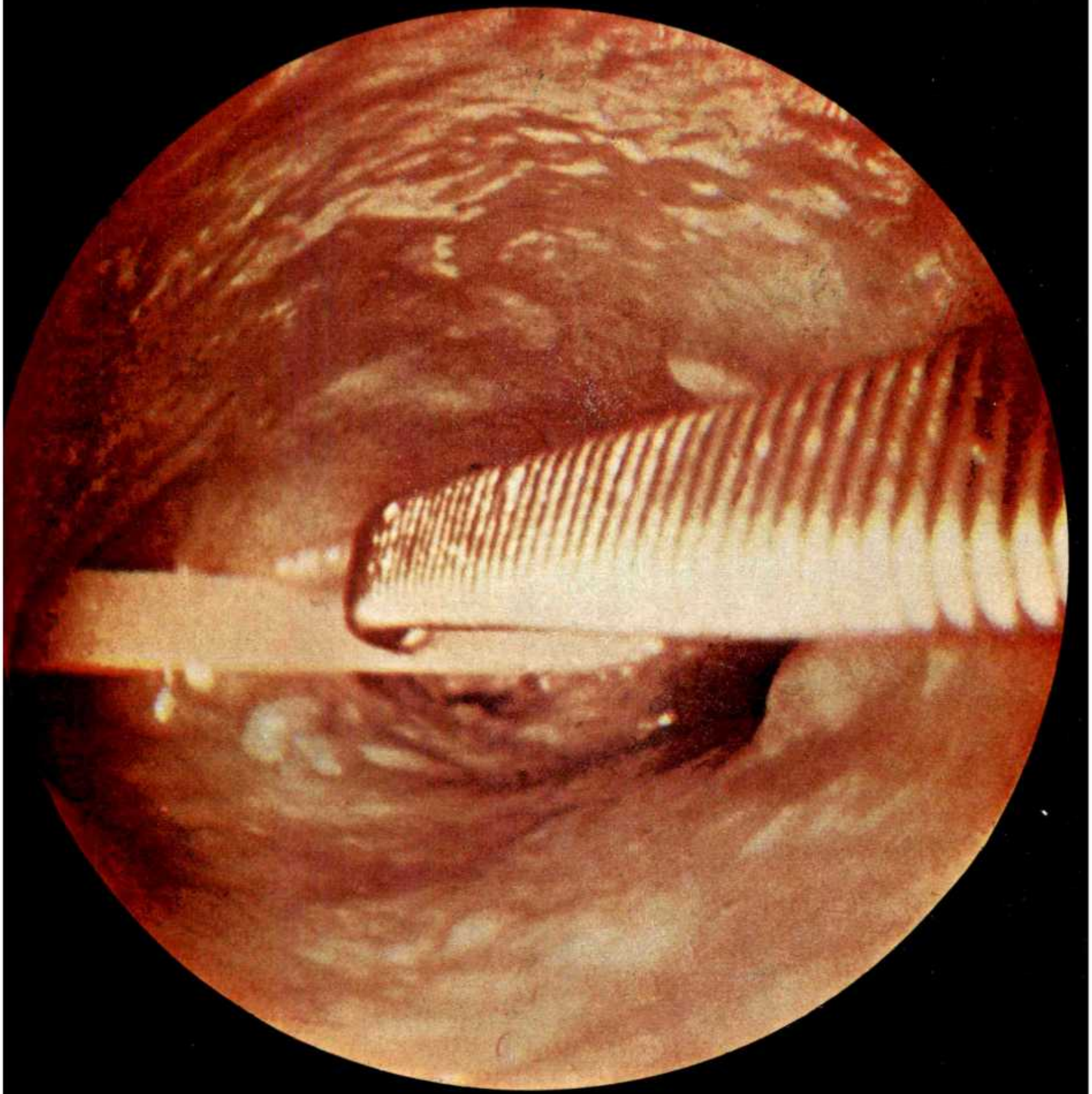

Gravigard in situ in
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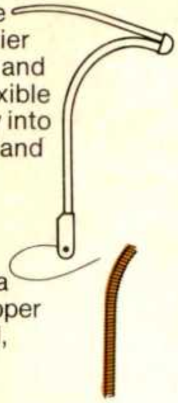
- Gravigard is the most advanced method of intrauterine contraception available and is only surpassed in efficiency by the combined pill.

| Method | Failure rates per 100 women years |
|---|---|
| No contraceptive[1] | **60** |
| Coitus interruptus[1] | **35** |
| Safe period[1] | **20** |
| Diaphragm[1] | **10** |
| Condom[1] | **10** |
| I.U.D.[2] | **5** |
| Progestogen-only pill[3] | **2·4** |
| **Gravigard**[3] | **1·1** |
| Combined pill[3] | **0·3** |

- Easy insertion is aided by the small size of the Gravigard carrier which also reduces discomfort and bleeding after insertion. The flexible 7 shape of Gravigard fits snugly into the uterus – so aiding retention and causing fewer expulsions.



- The plastic 7 merely acts as a carrier for the contraceptive copper which exerts a non-mechanical, local action on the uterus.

- Gravigard is easily and virtually painlessly inserted – the introducer tube diameter of 3 mm. is the smallest of any I.U.D.

Lippes 5·4 mm. ● (approx)

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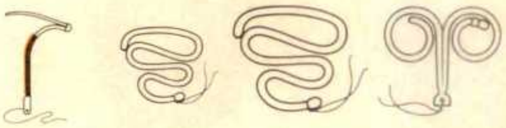
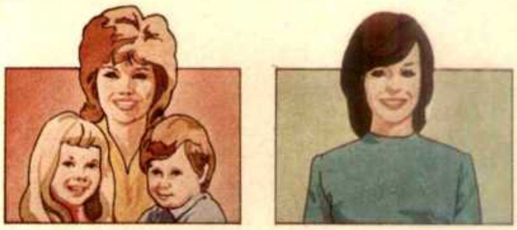
Uterine sound 3·5 mm. ●

**Gravigard 3·0 mm.** ●

- Gravigard, in contrast to other I.U.D.s., has a remarkably low incidence of removal because of pain and bleeding.



| **Gravigard**[2] | Lippes Loop C[4] | Lippes Loop D[4] | Saf-T-Coil[4] |
|---|---|---|---|
| **4·1** | 11·0 | 11·7 | 14·7 |



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Further information is available on request.

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[1] Human Reproduction and Society, Faber, 1974
[2] Brit. Med. J., 1974, **3,** 447–450
[3] Data on files of Searle Laboratories
[4] IPPF booklet "Intrauterine Contraception"
[5] Am. J. Obstet. Gynecol. 1973, **117,** 602

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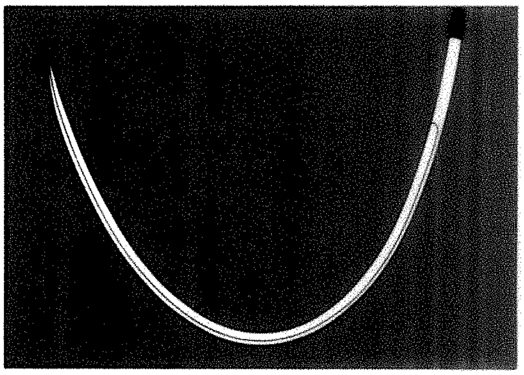

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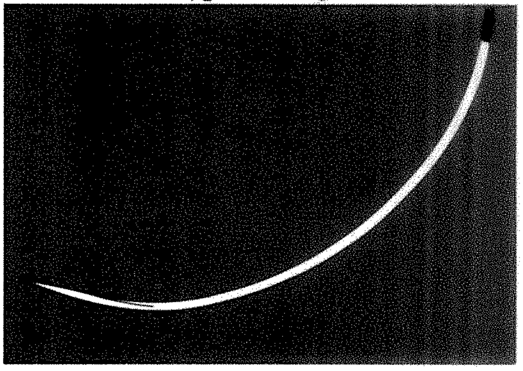

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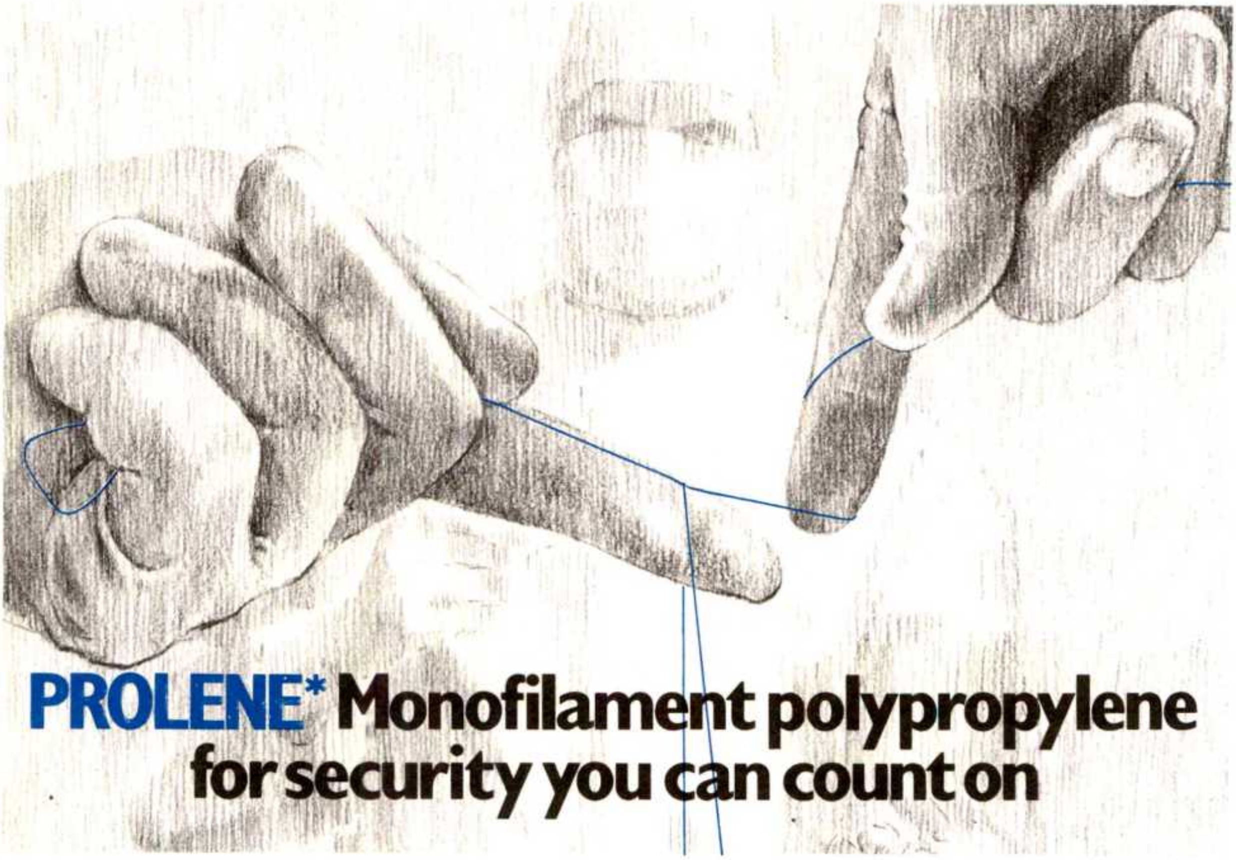
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J.Obstet.Gynaec. Brit. Cwlth. 1972. **79**. 517.

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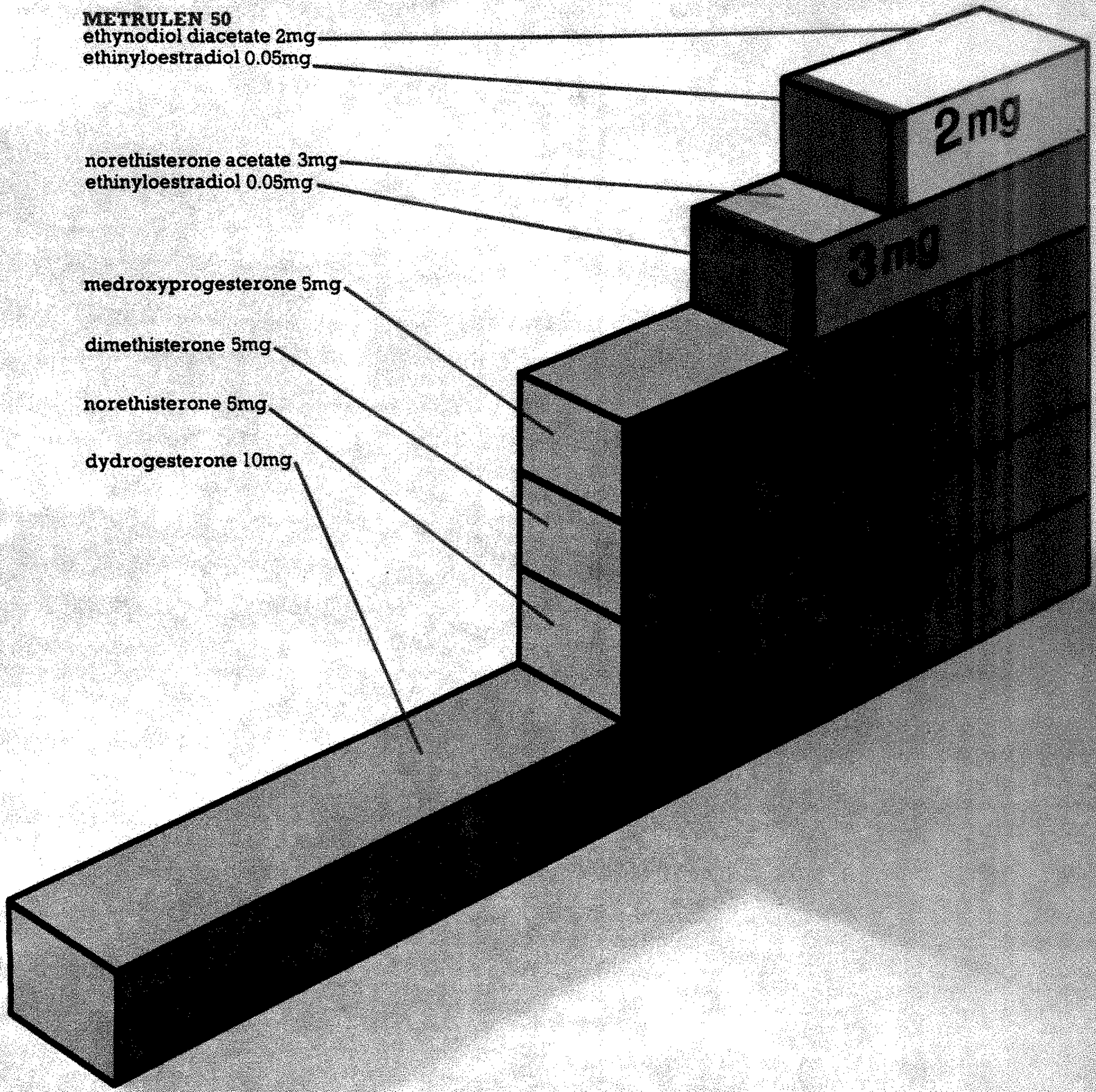

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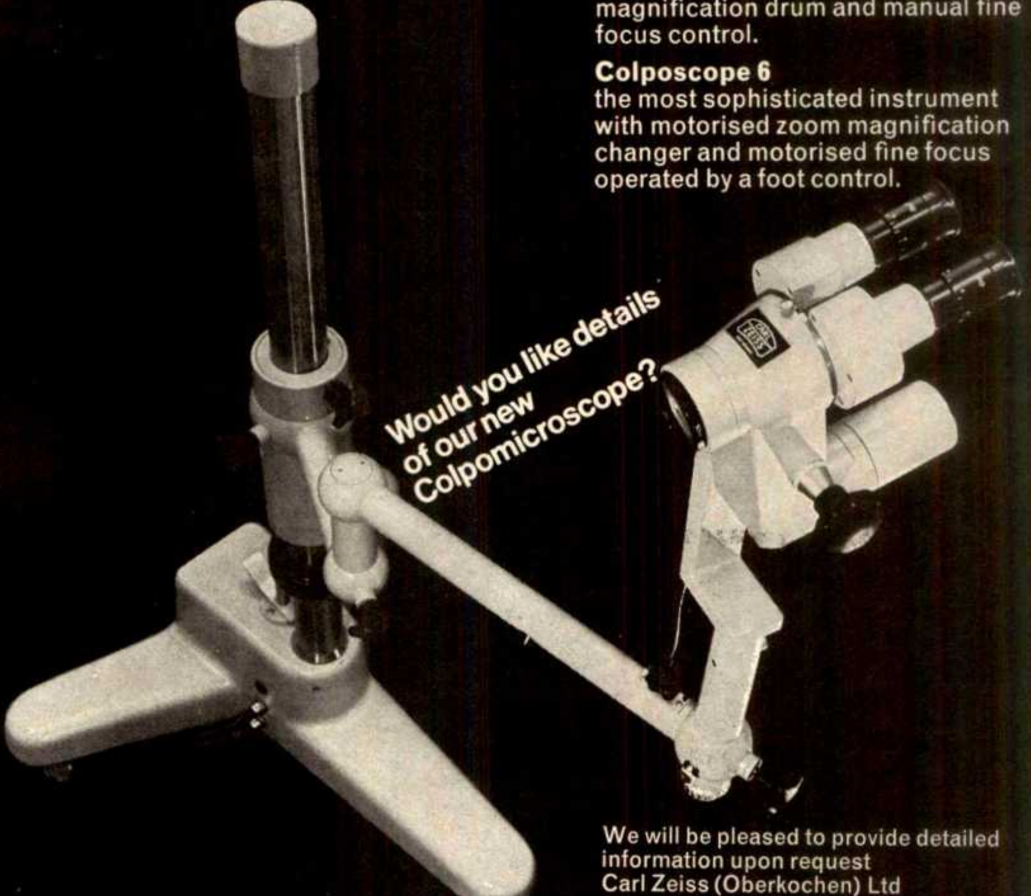

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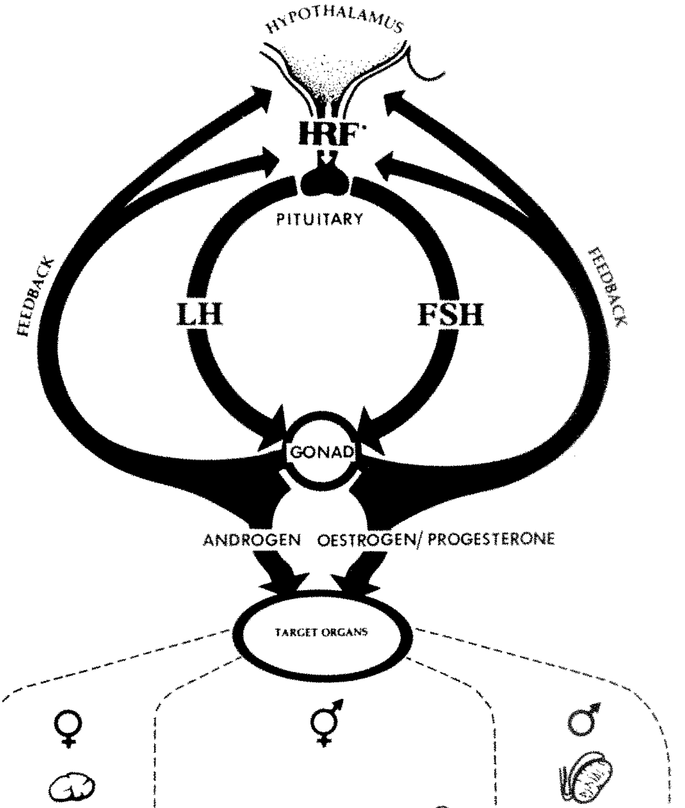

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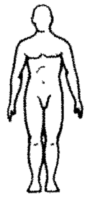
Sheehan's syndrome
Stein-Leventhal syndrome
Hypothalamic organic
  lesions
Hypogonadotrophic
    hypogonadism
  Primary amenorrhoea
  Secondary amenorrhoea
  —without galactorrhoea
    anorexia nervosa
    iatrogenic
    idiopathic
    psychogenic
    post-traumatic
    postpartum
  -with galactorrhoea
    iatrogenic
    idiopathic
    non-postpartum
    postpartum

**Male and Female**
Acromegaly
Cushing's syndrome
Prader-Willi syndrome
Simmonds' syndrome
Hypophysectomy
Pituitary ablation
Hypothalamic disorders
  Tumours
  Congenital lesions
    Kallmann's syndrome
Functional disorders
Panhypopituitarism
  Primary
  Secondary
    idiopathic or other
Hypogonadotrophic
  hypopituitarism
  Adult
  Pubertal



**Male**
Hypothalamic traumatism
Frohlich's syndrome
Klinefelter's syndrome
Hypogonadotrophic
  hypogonadism
  Primary
    Cryptorchism
  Secondary (with or
    without oligospermia
    or azoospermia)
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    systemic disease
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    idiopathic

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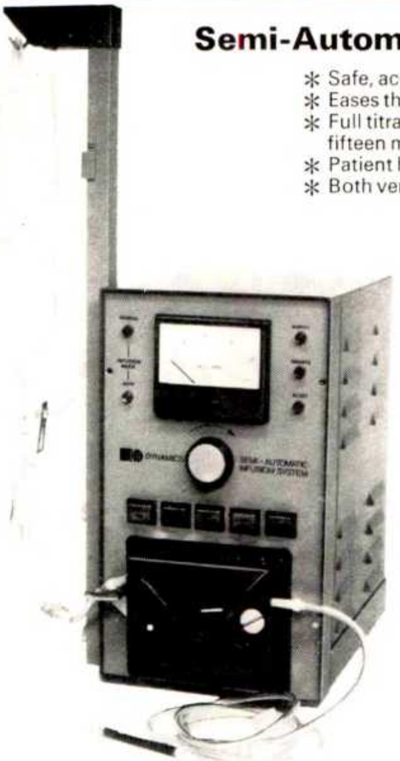

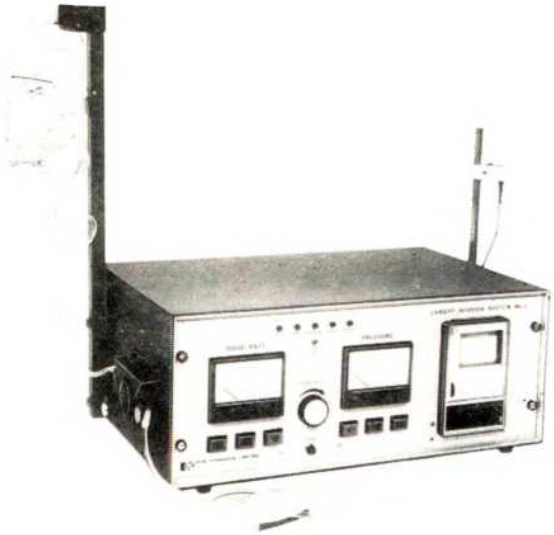

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3. Brit. J. Anaesth. (1974) 46:365-372.

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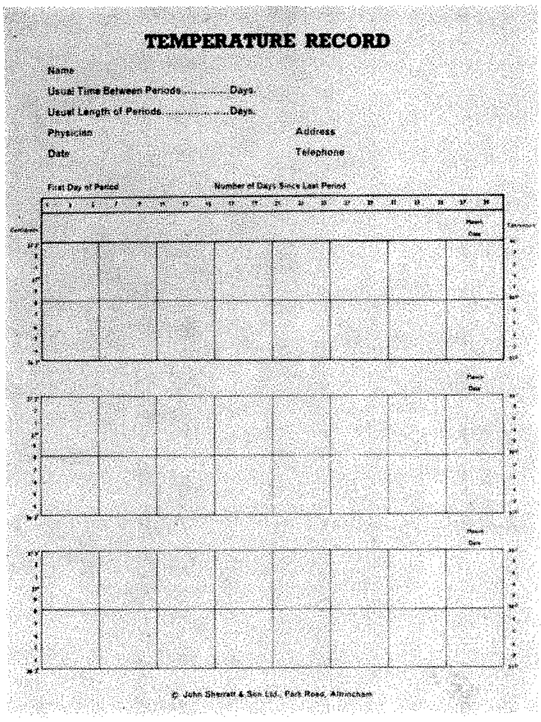

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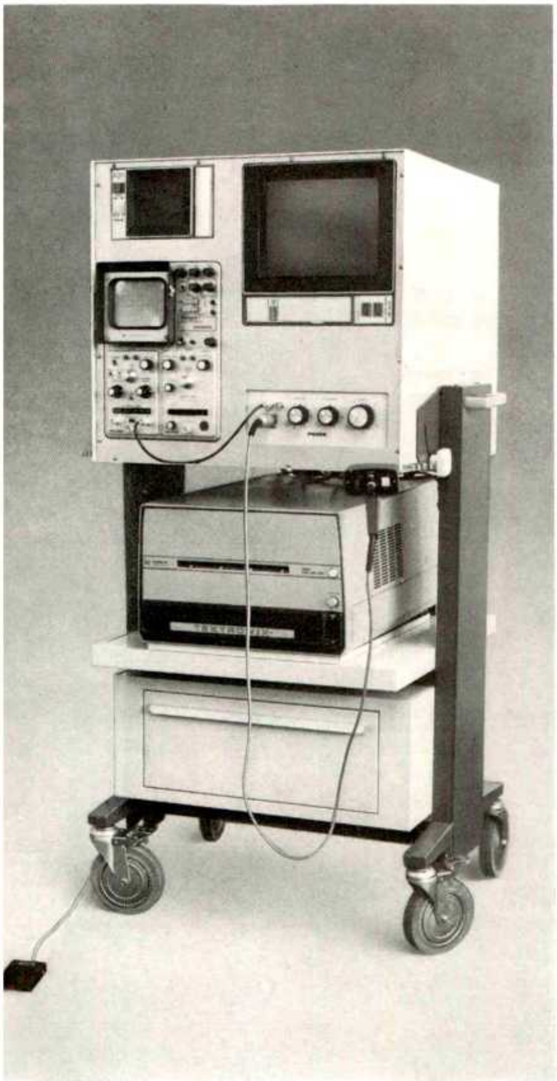

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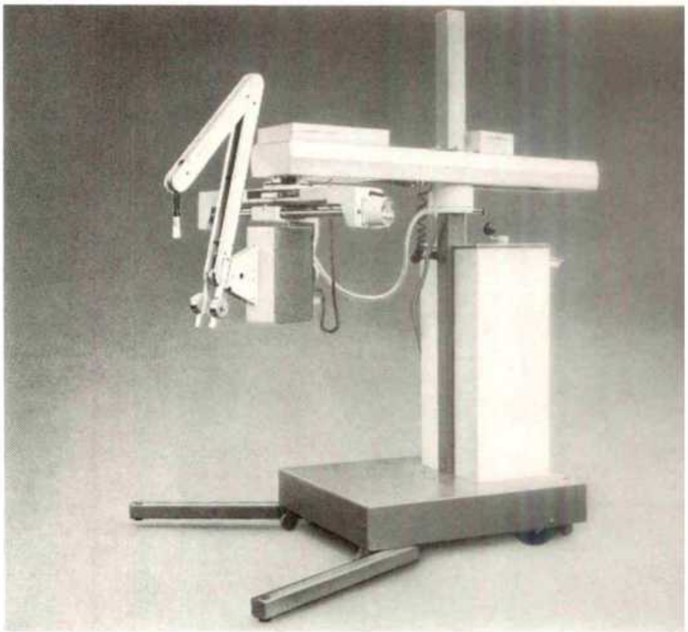

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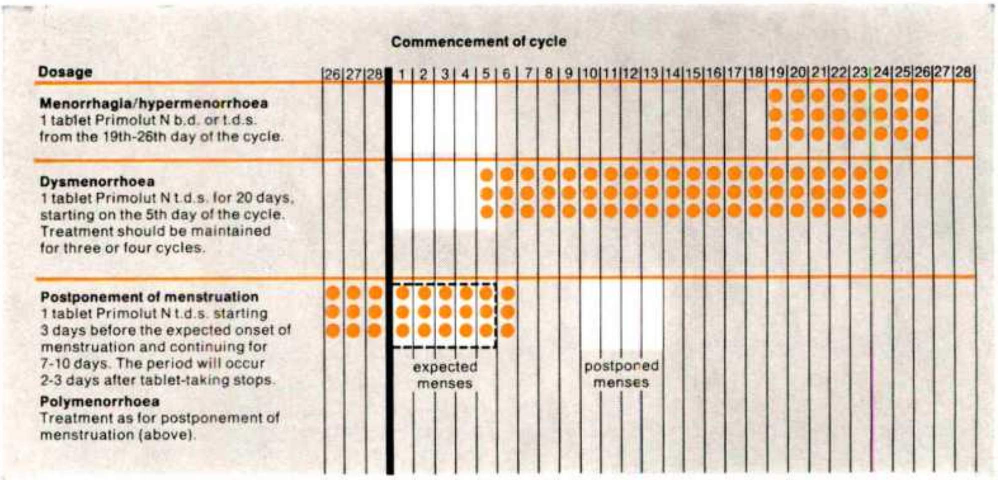

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British Journal of Obstetrics and Gynaecology

VOL. 82 No. 4 NEW SERIES APRIL 1975

# AMNIOTIC FLUID $_{\alpha1}$FETOPROTEIN IN THE DIAGNOSIS OF NEURAL TRACT MALFORMATIONS

BY

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## Summary

Amniotic fluid $\alpha_1$fetoprotein levels were determined in 400 pregnancies. The normal range for $\alpha_1$fetoprotein in amniotic fluid was defined by 350 samples from normal pregnancies. Elevated levels of $\alpha_1$fetoprotein were detected in 17 out of 18 pregnancies which gave rise to infants with neural tract abnormalities. The significance of this test in the antenatal diagnosis of neural tract abnormalities is discussed together with the possible reasons for "false negative" and "false positive" results. There were no complications in an individual series of 105 consecutive amniocenteses.

DURING its early months of life $\alpha_1$fetoprotein (AFP) is the major serum protein of the fetus; it is synthesized predominantly in the yolk sac and fetal liver (Abelev, 1971), but also, for a limited period, by cells in the gastrointestinal tract (Gitlin and Boesman, 1967). Serum levels in the fetus range from 66 μg./ml. at six weeks to a maximum of 3000 μg./ml. at 14 weeks (Gitlin and Boesman, 1966), and thereafter fall to a mean 100 μg./ml. at 40 weeks (Karlsson *et al.*, 1972).

AFP is present in the amniotic fluid, maximal concentrations being found in the 12 to 24 week gestation period. It is believed that AFP reaches the amniotic fluid via the fetal urine, and the amniotic fluid levels follow closely the pattern of fetal serum levels.

Brock and Sutcliffe (1972) first suggested that AFP might be a valuable diagnostic parameter in cases of anencephaly and myelomeningocoele. This initial observation has been followed by numerous reports (Lancet, 1974; Milunsky and Alpert, 1974; Randle and Cumberbatch, 1973) confirming their findings. It would be reasonable to say that it is now generally accepted that a raised amniotic fluid AFP level is diagnostic of a major neural tube malformation in early pregnancy, and that this diagnosis can often be confirmed by ultrasonic or radiographic examination. The source of the raised levels of AFP is still, however, in question. This report summarizes the findings in 400 pregnancies in which amniotic fluid AFP determinations were performed, and reports the results and complications in an individual series of 105 consecutive amniocenteses.

## METHODS

AFP was determined by single radial immunodiffusion in 2 per cent agarose gel containing 1.7 per cent monospecific rabbit antiserum to human $\alpha_1$fetoprotein (DAKO, Copenhagen). These immunodiffusion gels, produced in this laboratory, have an effective assay range of 1 to 90 $\mu$g./ml. Amniotic fluid samples were assayed undiluted, and diffusion was allowed to proceed overnight or for 18 hours. Standardization was achieved using Behringwerke AFP standard serum (OTOD 03). Results were further compared with the WHO standard serum (preparation 72/225); 1 $\mu$g. of the Behringwerke standard being equivalent to 1·54 I$\mu$. Units of of the WHO standard. Replicate analyses of a single sample showed a batch-to-batch and within batch coefficient of variation of less than 5 per cent.

### *Amniotic fluid samples*

Amniotic fluid samples were obtained by percutaneous amniocentesis from 400 pregnancies. These included pregnancies "at risk" of producing a neural tube abnormality or chromosomal abnormality; pregnancies about to be terminated on therapeutic grounds; and a miscellaneous group which included rhesus isoimmunization and amniocentesis to assess fetal maturity. A total of 350 samples was considered normal in that the fetus, on delivery, showed no evidence of neural tube malformation, was of a normal chromosomal karyotype, showed no evidence of rhesus isoimmunization and was of normal maturity for the gestational age.

Included in the total series is a consecutive series of 105 patients who underwent amniocentesis between 16 and 20 weeks gestation. The amniocentesis was performed primarily for AFP estimation in 63 patients (60 per cent) who had previously been delivered of a fetus with a neural tract defect, either anencephaly or spina bifida cystica. The remaining 42 patients (40 per cent) had either been delivered of a fetus with a chromosomal abnormality, were at risk of such a condition, or had been exposed to radiation or drugs early in pregnancy. The prime examination in these cases was amniotic cell culture and karyotype, but AFP determinations were performed in addition.

### *Amniocentesis*

Placental location prior to amniocentesis was not attempted in all cases. Prior to 16 weeks gestation it was found that an anterior placenta could not be avoided and that the most successful results were obtained by passing the needle at right angles to the longitudinal axis of the uterus, at a point in the midline just above the symphysis pubis. After 16 weeks gestation placental location by ultrasound B scan was employed; in all cases the fetal heart was heard before and after the procedure using the Sonicaid apparatus.

Amniocentesis was performed using the smallest possible needle. The most suitable was found to be a 5 cm. 21-gauge hypodermic needle, alternatively a 12 cm. 19-gauge spinal needle. Only two attempts to obtain amniotic fluid were made in each case, and always through the same point in the abdominal wall, If this failed, the patient was asked to attend again after 7 to 14 days, and a return visit was necessary in 12 patients. In five of these amniotic fluid was obtained on the repeat amniocentesis, but in seven none was obtained and attempts at antenatal diagnosis were abandoned. In all patients undergoing repeat amniocentesis, placental location was performed and an attempt made to identify a clear zone. All seven patients in whom antenatal diagnosis was abandoned were delivered of normal, healthy, infants.

### *Haemorrhage and contamination of amniotic fluid*

Maternal venous blood samples were taken prior to, and three minutes after, amniocentesis in all patients, whether the procedure was successful or not. The sequestrene sample was examined for the presence of fetal erythrocytes using a modified Kleihauer technique. After denaturation, 50 low power fields in each blood film were examined; in eight cases fetal erythrocytes were found in the post-amniocentesis sample. None of these patients was rhesus negative, and only one was in the group between 12 and 14 weeks gestation.

The amniotic fluid sample was also examined for blood contamination. In each case the first 1 to 2 ml. aspirated was kept separate from the sample for analysis. Blood contamination was present in this initial sample in 15 cases; the blood was of maternal origin in ten cases and of fetal origin in the remaining five.

## RESULTS

The results of AFP determinations in the amniotic fluid from 350 normal pregnancies are shown in Table I. There is a significant fall in AFP level during the second trimester.

Table II shows the AFP results in 18 pregnancies which gave rise to fetuses with neural tract abnormalities. These results are shown in Figure 1 in relation to the upper limit of the "normal" range, defined as the mean +2 log SD of the normal pregnancy series taken from Table I.

In five patients there was significant fetal haemorrhage into the amniotic fluid. In these cases the AFP concentrations were above the upper normal limit, 36 to 47 μg./ml. at 14 to 16 weeks gestation, but in one case repeat amniocentesis two weeks later showed a normal AFP value.

In the consecutive series of 105 amniocentesis, four patients were shown to have elevated amniotic fluid AFP levels (Table II, patients Nos. 4, 15, 16 and 17). All the normal pregnancies in this series continued relatively uneventfully following amniocentesis. The eventual outcome of the pregnancies included 99 normal live infants, four fetuses with neural tract anomalies, and two neonatal deaths. The two neonatal deaths unrelated to neural tube abnormality occurred in an infant with respiratory distress following Caesarean section

TABLE I

*Amniotic fluid AFP concentrations (μg./ml.) in 350 normal pregnancies*

| Duration of gestation (weeks) | n | Mean | Range |
|---|---|---|---|
| 11–12 | 5 | 24·4 | 9·9 — 47·8 |
| 13–14 | 38 | 23·3 | 13·1 — 41·4 |
| 15–16 | 129 | 18·3 | 9·5 — 35·2 |
| 17–18 | 102 | 14·6 | 6·4 — 33·3 |
| 19–20 | 30 | 10·2 | 4·1 — 25·4 |
| 21–25 | 16 | 7·3 | 3·8 — 13·8 |
| 26–30 | 9 | 6·0 | 3·7 — 9·5 |
| 31–35 | 8 | 2·6 | 0·5 — 7·2 |
| 36–40 | 15 | 1·7 | 0·4 — 5·0 |

TABLE II

*Amniotic fluid AFP values in pregnancies resulting in neural tract abnormalities*

| Patient No. | Duration of gestation (weeks) | AFP (μg./ml.) | Pregnancy outcome |
|---|---|---|---|
| 1 | 36 | 36 | Anencephaly |
| 2 | 37 | 9 | Anencephaly and spina bifida |
| 3 | 26 | 81 | Anencephaly |
| 4 | 20<br>22 | 250<br>190 | Anencephaly: terminated at 22 weeks |
| 5 | 36 | 7 | Anencephaly |
| 6 | 37 | 62 | Anencephaly |
| 7 | 35 | 29·5 | Anencephaly: induced at 35 weeks |
| 8 | 31 | 70 | Anencephaly: spontaneous delivery at 31 weeks |
| 9 | 36 | 22 | Anencephaly: spontaneous delivery at 36 weeks |
| 10 | 32 | 61·5 | Anencephaly |
| 11 | 16 | 600 | Anencephaly: hysterotomy termination at 18 weeks |
| 12 | 30 | 22 | Anencephaly |
| 13 | 14<br>16 | 680<br>900 | Anencephaly: terminated at 19 weeks |
| 14 | 13 | 382 | Spina bifida and cervical myelomeningocoele. Terminated at 15 weeks |
| 15 | 16<br>18<br>34 | 92<br>100<br>16 | Spina bifida and thoraco-lumbar myelomeningocoele: stillbirth following induction at 37 weeks |
| 16 | 16 | 248 | Spina bifida and myelomeningocoele: spontaneous abortion at 19 weeks |
| 17 | 20 | 100 | Spina bifida and hydrocephalus: spontaneous delivery at 32 weeks |
| 18 | 18 | 16 | Spina bifida and "closed" lumbar meningocoele. Live birth. |

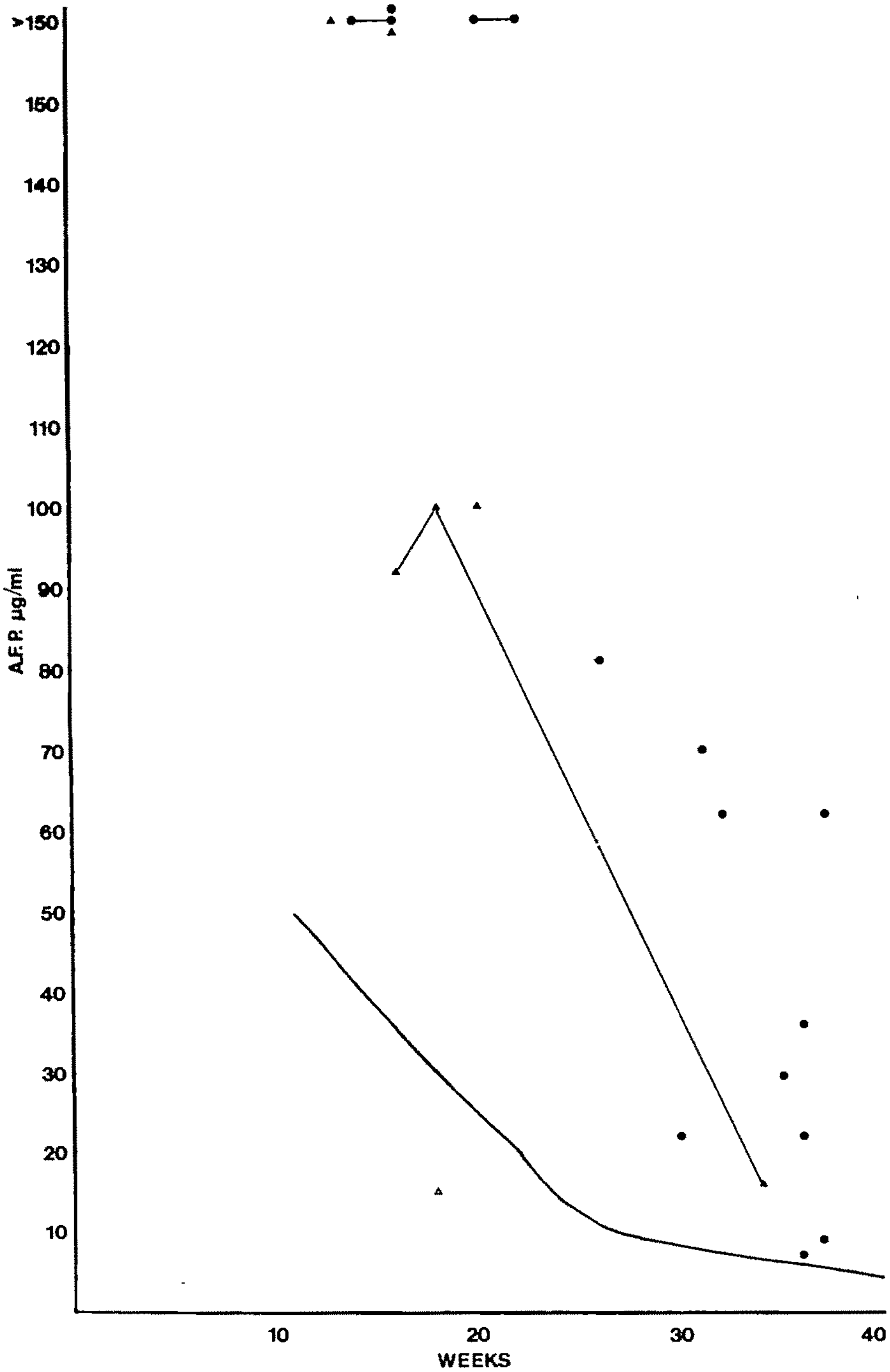

Fig. 1

AFP levels in major neural tract malformations. Anencephaly ●; Myelomeningocoele ▲ "open" △ "closed". Connected symbols represent repeat values. The solid line represents the upper normal limit for AFP in amniotic fluid (mean +2 log SD).

for pre-eclampsia and in an infant with a large diaphragmatic hernia.

## DISCUSSION

Since the first indication of the significance of amniotic fluid AFP in the diagnosis of neural tract malformations (Brock and Sutcliffe, 1972) there have been a number of confirmatory reports (Leading Article, 1974; Milunsky and Alpert, 1974; Randle and Cumberbatch, 1973). One striking feature appears from all the major published reports: whereas three separate methods have been employed in the estimation of AFP, namely, radial immunodiffusion, electroimmunoassay and radioimmunoassay, the normal ranges are essentially similar. All reports agree on the maximal levels occurring at 12 to 14 weeks gestation and falling thereafter to low levels after 25 to 26 weeks gestation. Minor differences that do exist between reports can now be resolved if general reference is made to the WHO standard preparation.

This report confirms the feasability of diagnosing neural tract malformations at a stage in pregnancy when termination can be offered. Sellar *et al.* (1974) reported three cases of chromosomal aberration being associated with raised AFP levels, but the karyotype in all cases in this series, in which it was examined, was normal. With the exception of the cases of neural tract malformation and of fetal haemorrhage, there were no other instances of raised AFP levels. The volume of fetal blood necessary to produce an elevation in the AFP can be calculated (Ward and Stewart, 1974), but the most important test in this respect is the Kleihauer test on the amniotic fluid as well as the maternal blood before and after amniocentesis. It is of the utmost importance that fetal blood contamination of the amniotic fluid sample can be identified as even small degrees of contamination can lead to marked changes in the AFP levels.

The series included one "false negative"; this child had a small lumbar myelomeningocoele with full thickness epidermalization and without CSF communication. The AFP result in this case was within the accepted normal range, adding further confirmation to the thesis that this technique will not be of value in the diagnosis of "closed" lesions.

In the personal series of 105 consecutive cases, using the amniocentesis technique described, despite the seven cases in which fluid was not obtained, and despite the 15 per cent of blood-stained samples, no cases of rhesus sensitization occurred and there were no cases of abortion or premature labour. The one spontaneous abortion (patient No. 16) followed 48 hours after amniography and it is felt that this cannot be directly attributed to the original amniocentesis which was performed seven days before the second procedure.

The value of amniocentesis and AFP determination in this high-risk group of patients is not so much to identify the abnormalities as to reassure those mothers who have had an abnormal baby that it is possible to identify future abnormalities at a stage when termination can be offered. The criticism of the method rests on the morbidity of the procedure of amniocentesis, but the results from this series show that there need not be a significant morbidity and that the technique can be offered to all those mothers "at risk".

## ACKNOWLEDGEMENTS

We thank our Consultant colleagues in Doncaster, Gloucester, Grimsby, Leicester, Manchester, Mansfield, Nottingham, Peterborough, Scunthorpe and Sheffield for their co-operation in providing the amniotic fluid samples, for referring patients to us and for their permission to include details of their various patients in this series.

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*British Journal of Obstetrics and Gynaecology*
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# IMMUNOGLOBULIN M IN HUMAN AMNIOTIC FLUID AND ITS POSSIBLE ASSOCIATION WITH NEURAL-TUBE MALFORMATIONS

BY

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## Summary

Immunoglobulin M (IgM) was found in the amniotic fluid from two fetuses with neural tube defects but not in that from normal fetuses. The possible associations of IgM with abnormalities of the central nervous system are discussed.

THE quantitation of α-fetoprotein (AFP) in amniotic fluid has enabled neural tube abnormalities to be detected antenatally with a high degree of specificity (Brock and Sutcliffe, 1972; Lorber *et al.*, 1973; Nevin *et al.*, 1973; Seller *et al.*, 1973; Lindsey *et al.*, 1973). However, the availability of additional indicators to couple with AFP estimation is highly desirable in view of the responsibility involved in terminating a pregnancy. The present preliminary report concerns the results of screening amniotic fluid for a number of other proteins in addition to AFP.

## MATERIALS AND METHODS

AMNIOTIC fluid specimens were obtained from 11 patients and after centrifugation the supernatant was stored at −20 °C. All specimens except one (patient G) were sent to the Department of Human Genetics, University of Newcastle upon Tyne, for antenatal diagnosis of genetic disorders. Following termination by hysterectomy for multiple fibroids in patient G, the fetus proved to have a lumbar myelomeningocoele; amniotic fluid was taken by aspiration from the intact amniotic sac. Transabdominal amniocentesis was used to obtain amniotic fluid from patient K at 16 weeks and on subsequent termination the fetus was observed to have a spina bifida. The remaining nine patients had uneventful pregnancies following amniocentesis.

Quantitative estimations of albumin, immunoglobulin A (IgA), IgG, IgM, α-2-macroglobulin and AFP in the amniotic fluid were made by means of the single radial immunodiffusion technique (Mancini *et al.*, 1965) using commercially available plates (Behringwerke AG).

## RESULTS

The quantitative estimations of the proteins are summarized in Table I. Two specimens, G and K, had concentrations of AFP of 153 and 330 μg./ml. respectively. Albumin was detectable in all specimens with a range of 24 to 386 mg./100 ml.; α-2-macroglobulin was not detected in any specimen. Immunoglobulin A was undetectable in three patients and IgG in one. Immunoglobulin M was present in the specimens G and K at levels of 7·1 and 6·9 mg./100 ml. respectively, while in the remaining specimens it was not detectable.

---

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TABLE I

*Concentration of Albumin, α2-Macroglobulin, IgA, IgG, IgM and AFP in amniotic fluid*

| Specimens | Albumin (mg./100 ml.) | 2-Macroglobulin (mg./100 ml.) | IgA (mg./100 ml.) | IgG (mg./100 ml.) | IgM (mg./100 ml.) | AFP (μg./ml.) |
|---|---|---|---|---|---|---|
| A | 316 | N.D. | 2·0 | 32 | N.D. | 25 |
| B | 386 | N.D. | 0·8 | 26 | N.D. | 25 |
| C | 112 | N.D. | N.D. | 6·5 | N.D. | 17 |
| D | 322 | N.D. | 0·8 | 39 | N.D. | 19 |
| E | 380 | N.D. | N.D. | 32 | N.D. | 24 |
| F | 360 | N.D. | 1·3 | 35 | N.D. | 19 |
| G | 24 | N.D. | N.D. | N.D. | 7·1 | 153 |
| H | 385 | N.D. | 0·1 | 30 | N.D. | 29 |
| I | N.T. | N.T. | N.T. | N.T. | N.D. | 22 |
| J | N.T. | N.T. | N.T. | N.T. | N.D. | 35 |
| K | 350 | N.D. | 0·6 | 32 | 6·9 | 330 |

N.D. = Not Detectable; N.T. = Not Tested
Albumin, α2-macroglobulin and AFP measured on M Partigen plates;
IgA and IgM measured on L. C.-Partigen plates; IgG measured on Tri-Partigen plates.

## DISCUSSION

Of the proteins studied other than AFP only IgM showed any increased level associated with neural tube defect. It is known that the human fetus can synthesize IgM in the liver from as early as 10½ weeks (Gitlin and Biasucci, 1969), but that the levels of immunoglobulins, mainly IgM, produced by the fetus remain very low until after birth (van Furth *et al.*, 1965). However, the fetus appears to be immunologically competent from about 20 weeks as intrauterine infections such as rubella lead to an increased IgM response after this time (Alford, 1965; Bellanti *et al.*, 1965).

IgM was not detectable in the amniotic fluid of the normal fetuses in the present series. This is in agreement with the work of Gitlin and Biasucci (1969) who were unable to detect IgM in amniotic fluid of 6½ to 38 weeks gestation in spite of 20 to 40 times concentration. Similarly, Fischbacher and Quinlivan (1970) and Curl (1971) failed to detect IgM in amniotic fluid at term.

The elevated IgM levels found in amniotic fluid from fetuses with neural tube defects were an unexpected finding for which there is no immediate explanation. It would appear unlikely that at 12 to 16 weeks the very low concentration of IgM in the serum of normal fetuses (van Furth *et al.*, 1965) would be sufficient to account for the levels found in amniotic fluid in the cases with open neural tube defects. This, however, might not be the case if IgM levels are raised in the serum of such fetuses. At present we have no information as to whether this is so or not.

Maternal blood can be excluded as a source of IgM because α-2-macroglobulin was not detected, and further, specimen G was obtained by aspiration from an intact amniotic sac after being carefully cleaned.

There remains the possibility of local production of immunoglobulin within the central nervous system. Evidence for this comes from the observation that raised levels of IgM occur in the cerebrospinal fluid of patients with sleeping sickness (Greenwood and Whittle, 1973). Similarly, Lord *et al.* (1973) found a high level of IgM in the cerebrospinal fluid of a patient suffering from a combined immunodeficiency disease who had developed a chronic progressive encephalopathy. They concluded that the synthesis of IgM was restricted to lymphoid cells in the central nervous system because no IgM was detected in the serum.

In view of these observations, it is reasonable to suppose that the lymphoid cells of the fetal central nervous system might produce IgM if exposed to antigenic stimulation, an occurrence which does not normally take place during early intrauterine life. Thus, if the amniotic fluid contains substances which are antigenic to the fetus, direct contact of this fluid with the central nervous system may give rise to a local production of IgM.

In conclusion, these observations suggest that the presence of IgM in the amniotic fluid of two fetuses with open neural tube defects was probably the result of a direct stimulus to the lymphoid tissue of the central nervous system by foreign antigens in the amniotic fluid. Further work is in progress to confirm the validity of the present observations.

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# NEONATAL HYPERBILIRUBINAEMIA FOLLOWING THE USE OF OXYTOCIN IN LABOUR

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## Summary

A prospective study of 1353 labours and the relevant newborn failed to reveal any significant difference between the incidence of neonatal hyperbilirubinaemia (defined as a level of 12 mg. or more per 100 ml.) following spontaneous labour, and after labour induced or accelerated by oxytocin. The incidence of unexplained neonatal hyperbilirubinaemia after spontaneous labour was 6·3 per cent. Following induced labour however there was a highly significant ($P<0·001$) association between the mean total dose of oxytocin used for induction and the incidence of neonatal hyperbilirubinaemia. The proportion of babies who developed hyperbilirubinaemia increased in direct relation to the total dose of oxytocin used for the induction. In this series the incidence of hyperbilirubinaemia increased sharply when the total dose of oxytocin exceeded 20 units as it did in 12 per cent of induced labours. No association was demonstrated between neonatal hyperbilirubinaemia and birthweight, or duration of spontaneous labour. When labour was induced, however, the proportion of newborn babies with hyperbilirubinaemia increased with the duration of labour. The significance of these findings is discussed.

SEVERAL reports have expressed concern at the high incidence of neonatal jaundice for which there is no known cause (Ghosh and Hudson, 1972; Norman *et al.*, 1972; O'Driscoll, 1972). At the Liverpool Maternity Hospital 5·4 per cent of newborn babies developed hyperbilirubinaemia (with serum total bilirubin level of 12 mg. or more per 100 ml.), during 1973.

It has been suggested that the use of oxytocin in the management of labour may be the causative factor (Ghosh and Hudson, 1972; Davies *et al.*, 1973). The aim of this study was to determine whether there is an association between the administration of oxytocin to mothers during labour and the subsequent occurrence of neonatal hyperbilirubinaemia.

## PATIENTS AND METHODS

A total of 1393 consecutive patients who were more than 37 weeks pregnant by dates and who were delivered vaginally at the Liverpool Maternity Hospital was studied prospectively. They were divided into three groups according to the type of labour: (1) spontaneous, (2) accelerated with oxytocin, and (3) induced by forewater amniotomy and simultaneous intravenous infusion of oxytocin in escalating doses.

Intravenous infusions of oxytocin were controlled by IVAC constant infusion pumps. The eventual rate of infusion was titrated against uterine response. The drip rates and the duration of labours were recorded and the total dose of oxytocin administered during each patient's labour was calculated.

All babies were given an intramuscular injection of 1 mg. of phytomenadione within 24 hours of birth. The neonatal serum bilirubin concentration was estimated by the use of a direct reading bilirubinometer (American Optical Corporation) in capillary blood obtained by heel prick. The serum bilirubin concentration in each of the babies was measured on the third day of life, and also on the sixth day in all but 475 (35 per cent) for whom earlier discharge from hospital had been arranged. A baby whose serum bilirubin concentration reached or exceeded 12 mg. per 100 ml. on the third or the sixth day of life was considered to have hyperbilirubinaemia. Babies discharged on the third day of life from hospital were not followed up further, but no baby was allowed home before the sixth day if the serum bilirubin level on the third day was 9 mg. per 100 ml. or more.

## RESULTS

Of the 1393 patients initially included in the study, 40 were subsequently excluded because their babies weighed less than 2·5 kg., or because they possessed erythrocyte antibodies which could possibly have been responsible for haemolysis.

Amongst the remaining 1353 patients the onset of labour was spontaneous in 687 but in 149 of these labour was subsequently accelerated with oxytocin. There were 666 patients in whom labour was induced artificially by forewater amniotomy and simultaneous intravenous oxytocin.

The total serum bilirubin concentrations in babies born after a spontaneous labour is shown in Figure 1. On the third day of life the largest proportion of babies had a bilirubin concentration of 7 to 8 mg. per 100 ml. On the sixth day the highest proportion of babies had a bilirubin concentration of 4 to 5 mg. per 100 ml. A similar distribution of bilirubin concentrations occurred in babies born after labours which were induced or accelerated (Fig. 2). Because some babies were discharged early from hospital the absolute number in each bilirubin class is less on the sixth day of life than on the third day.

The highest bilirubin level recorded was 18·5 mg. per 100 ml. Phototherapy and phenobarbitone were used in a small number of babies when bilirubin concentrations exceeded 12 mg. per 100 ml. but exchange transfusion was never necessary.

Table I shows that the mean bilirubin concentration of babies born after induced labour did not differ significantly from the mean con-

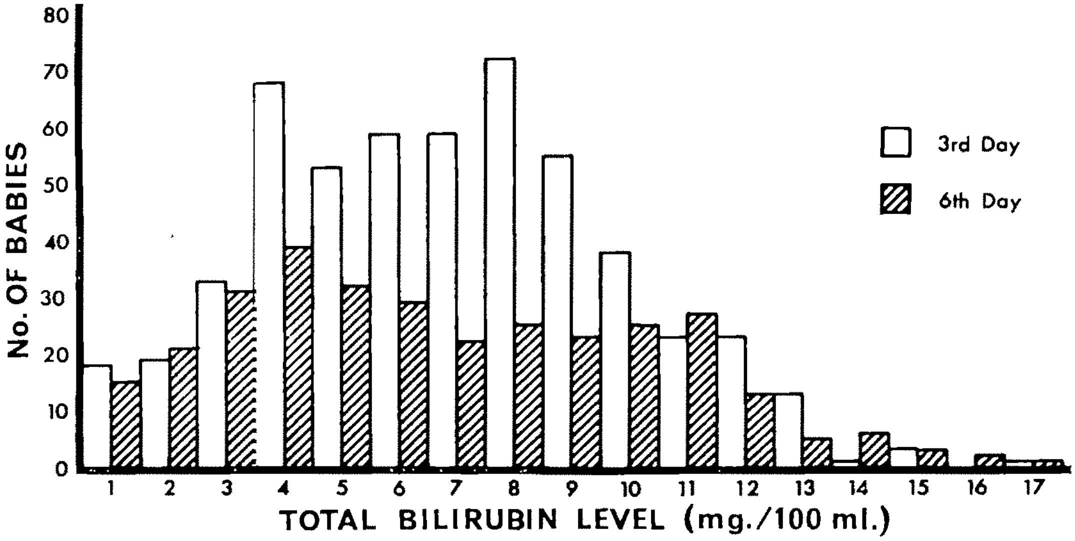

FIG. 1

Histogram showing the frequency distribution of bilirubin concentrations in babies following spontaneous labours.

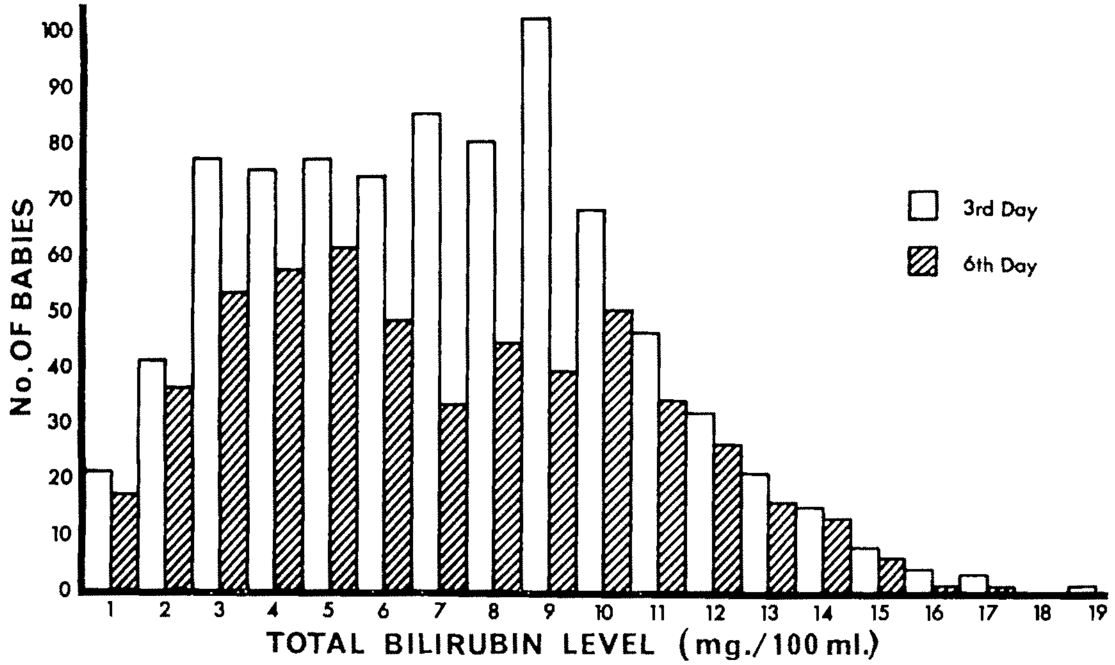

FIG. 2
Histogram showing the frequency distribution of bilirubin concentrations in babies following induced or accelerated labour.

TABLE I
*Mean bilirubin concentrations on the third and sixth days of life in babies following spontaneous, accelerated and induced labours*

| Type of labour | No. of babies | Mean bilirubin concentration (mg./100 ml.) | |
|---|---|---|---|
| | | Third day | Sixth day |
| | | Mean±SD | Mean±SD |
| *Spontaneous* | | | |
| (No oxytocin) | 538 | 6·50±3·20 | 6 39±2 99 |
| (Accelerated with oxytocin) | 149 | 7·13±3·59 | 7 02±3·42 |
| *Induced* | 666 | 6·67±3·16 | 6·35±3·71 |
| Total | 1353 | | |

centration in babies born after spontaneous labour (*t* test; $P > 0 \cdot 6$) or from the mean concentration in babies born by accelerated labour (*t* test; $P > 0 \cdot 05$).

The incidence of babies with a serum bilirubin concentration of 12 mg. per 100 ml. or more is shown in Table II. After spontaneous labour without acceleration, it was 6·3 per cent. When the incidence following induced labour was compared with the incidence following spontaneous labour no significant difference was observed ($\chi^2$ : $P > 0 \cdot 10$). The incidence following induced and accelerated labour did not differ significantly either ($\chi^2$ : $P > 0 \cdot 80$).

TABLE II

*Incidence of hyperbilirubinaemia (12 mg./100 ml. or more) on the third or sixth day of life in babies following spontaneous, accelerated and induced labours*

| Type of labour | No. of babies | No. with bilirubin of 12 mg./100 ml. or more on the third or sixth day | | |
|---|---|---|---|---|
| *Spontaneous* | | | | |
| (No oxytocin) | 538 | 34 | 58 | (8·44%) |
| (Accelerated with oxytocin) | 149 | 24 | | |
| *Induced* | 666 | 55 | | (8·25%) |
| Total | 1353 | 113 | | (8·35%) |

TABLE III

*Incidence of neonatal hyperbilirubinaemia (bilirubin concentration of 12 mg. per 100 ml. or more) in relation to birthweight following spontaneous and induced labours*

| Birthweight (kg.) | Spontaneous labour | | Induced labour | |
|---|---|---|---|---|
| | Total No. of babies | No. of babies with bilirubin of 12 mg. per 100 ml. or more | Total No. of babies | No. of newborn with bilirubin of 12 mg. per 100 ml. or more |
| 2·5–3·0 | 127 | 13 (10·3%) | 129 | 12 (9·3%) |
| 3·1–3·5 | 243 | 8 (3·3%) | 300 | 29 (9·7%) |
| 3·6–4·0 | 143 | 10 (7·1%) | 184 | 12 (6·5%) |
| 4·1–4·5 | 25 | 3 (12%) | 47 | 2 (4·3%) |
| >4·5 | – | – | 6 | – |
| Total | 538 | 34 (6·3%) | 666 | 55 (8·25%) |

Breast feeding was commenced by 392 (28 per cent) of the mothers. Of the babies who developed hyperbilirubinaemia, 31·42 per cent were breast fed at least initially, compared with 27·44 per cent of babies who did not develop hyperbilirubinaemia. This difference is not significant ($\chi^2$ test : $P<0\cdot70$).

*Relationship between hyperbilirubinaemia and birthweight*

Table III shows the distribution of newborn babies with hyperbilirubinaemia against birthweight following spontaneous and induced labours. Chi-squared tests for trend failed to show any significant association between hyperbilirubinaemia and birthweight (spontaneous labour: $P>0\cdot80$; induced labour, $P>0\cdot10$).

*Relationships between oxytocin dosage and hyperbilirubinaemia*

The mean total dose of oxytocin employed to induce labours which resulted in normal babies and babies with hyperbilirubinaemia is shown in Table IV. The mean total dose of oxytocin received by the mother was only 9·91 units when the baby was normal, whilst mothers of babies with hyperbilirubinaemia received a mean dose of 18·77 units. This difference is highly significant ($t$ test : $P<0\cdot001$). The proportion of babies who developed hyperbilirubinaemia increased in direct relation to the total dose of oxytocin administered to induce labour (Table V) and this association is also highly significant (Wilcoxon's test of differences between mean ranks : $P<0\cdot001$).

Following accelerated labour normal babies

TABLE IV

*Incidence of neonatal hyperbilirubinaemia (bilirubin of 12 mg./100 ml. or more) on the third or sixth day of life in relation to the total dose of oxytocin received by the mothers in order to induce or accelerate labour*

| Neonatal bilirubin concentration (mg./100 ml.) | Induced labour | | Accelerated labour | |
|---|---|---|---|---|
| | No. of babies | Dose of oxytocin received by mothers (units) | No. of babies | Dose of oxytocin received by mothers (units) |
| | | Mean±SD | | Mean±SD |
| Less than 12 | 611 | 9·91±11·41 | 125 | 6·42±6·26 |
| 12 or more | 55 | 18·77±19·10 | 24 | 12·78±14·44 |
| Total | 666 | | 149 | |

TABLE V

*Incidence of neonatal bilirubin concentrations of 12 mg./100 ml. or more on the third or sixth days of life relative to the total dose of oxytocin employed to induce or accelerate labour*

| Dose of oxytocin received by mothers (units) | Induced labour | | Accelerated labour | |
|---|---|---|---|---|
| | No. of babies | No. of babies with bilirubin of 12 mg./100 ml. or more | No. of babies | No. of babies with bilirubin of 12 mg./100 ml. or more |
| <10 | 483 | 33 (6·8%) | 117 | 15 (12·8%) |
| 10–19 | 103 | 9 (8·7%) | 22 | 5 (22·7%) |
| 20–29 | 41 | 6 (14·6%) | 5 | 1 (20·0%) |
| >30 | 39 | 7 (18·0%) | 5 | 3 (60·0%) |
| Total | 666 | 55 (8·3%) | 149 | 24 (16·1%) |

were found when the mean dose of oxytocin given to the mother was 6·42 units. In babies with hyperbilirubinaemia the mother had received a mean dose of 12·78 units during labour. This difference is significant ($t$ test : $P<0{\cdot}05$). As shown in Table V, the proportion of newborn babies with hyperbilirubinaemia is only just significantly related to the total dose of oxytocin used to accelerate labour when examined by the $\chi^2$ test ($0{\cdot}02<P<0{\cdot}05$) and the association is not significant when the Fisher-Irwin "exact" test ($2P$ approximately 0·06) or Wilcoxon's test of the differences between mean ranks ($0{\cdot}1<P<0{\cdot}2$) is applied.

It will be noticed in Table V that only five patients received more than 30 units of oxytocin to accelerate labour. These five contribute disproportionately to the value of $\chi^2$ for the whole group, and if they are omitted from the Table there is no significant association ($\chi^2$ : $0{\cdot}3<P<0{\cdot}5$) between the dose of oxytocin used to accelerate labour and neonatal hyperbilirubinaemia.

### *Duration of labour*

No significant relationship was found between the duration of labour (Table VI) and the incidence of hyperbilirubinaemia when labour was spontaneous (Wilcoxon's rank test: $0{\cdot}20<P<0{\cdot}30$; $\chi^2$ test : $0{\cdot}30<P<0{\cdot}50$), or accelerated (Wilcoxon's rank test: $0{\cdot}10<P<0{\cdot}20$; $\chi^2$ test : $0{\cdot}20<P<0{\cdot}30$). However, when labour was induced artificially, there was a highly significant association between the increasing duration of labour and the subsequent development of neonatal hyperbilirubinaemia (Wilcoxon's rank test: $P<0{\cdot}001$; $\chi^2$ test : $P<0{\cdot}01$).

## DISCUSSION

In contrast to the report by Davies *et al.* (1973)

TABLE VI

*Incidences of newborn babies with bilirubin concentrations of 12 mg./100 ml. or more on the third or sixth day of life following spontaneous, accelerated and induced labours of varying duration*

| | Spontaneous labour | | Accelerated labour | | Induced labour | |
|---|---|---|---|---|---|---|
| Duration of labour (hours) | Total No. | No. of babies with bilirubin concentration of 12mg./100ml. or more | Total No. | No. of babies with bilirubin concentration of 12mg./100ml. or more | Total No. | No. of babies with bilirubin concentration of 12mg./100ml. or more |
| <5 | 190 | 15 | 48 | 4 | 348 | 20 |
| 5–9·9 | 236 | 15 | 54 | 13 | 235 | 21 |
| 10–14·9 | 91 | 4 | 31 | 5 | 63 | 9 |
| >15 | 21 | 0 | 16 | 2 | 20 | 5 |
| Total | 538 | 34 | 149 | 24 | 666 | 55 |

we have been unable to show a significant difference between the *mean* bilirubin concentrations of babies born after induced or spontaneous labours. But by examining the incidence of hyperbilirubinaemia following induced and accelerated labour it was found that mothers whose babies developed hyperbilirubinaemia received a significantly higher mean total dose of oxytocin than mothers whose babies were not so affected. When labour was induced, oxytocin also appeared to influence neonatal hyperbilirubinaemia in a manner which was dose dependent. The incidence of hyperbilirubinaemia rose sharply when the total dose of oxytocin administered exceeded 20 units as it did in 12 per cent of the induced labours.

The incidence of neonatal hyperbilirubinaemia was found to increase as the duration of induced labour increased. No similar relationship was discovered between the incidence of neonatal hyperbilirubinaemia and the duration of spontaneous labour. It seems unlikely therefore that neonatal hyperbilirubinaemia is directly related to either the duration of labour or to the use of analgesic drugs. These observations could, however, be explained on the grounds of an increasing total dose of oxytocin associated with increasing duration of induced labour.

The acceleration of spontaneous labour with oxytocin was found not to have any significant dose dependent relationship to neonatal hyperbilirubinaemia in this study. Therefore, the occurrence of hyperbilirubinaemia in the offspring of women whose labours were induced by amniotomy and oxytocin may reflect immaturity of the hepatic enzyme system to deal with the bilirubin load. Although babies delivered prior to 37 weeks by dates were excluded from the study, as an added precaution babies of low birthweight were also excluded. The absence of a significant association between neonatal hyperbilirubinaemia and birthweight of the remaining babies supports the view that unexpected prematurity did not seriously bias the results. The absence of any meaningful association between increasing doses of oxytocin and neonatal hyperbilirubinaemia in accelerated labours whilst such an association was demonstrable in induced labours suggests that the mode of onset of labour may also be of importance. Despite these associations, the influence of both artificial induction of labour and the use of oxytocin appears to be minimal, there being no significant difference in the incidence of hyperbilirubinaemia following induced or spontaneous labour. The frequency of breast feeding was approximately equal amongst "normal" and hyperbilirubinaemic babies but it is appreciated that bias away from breast feeding may have occurred if babies were considered to be unsuitable for other reasons.

This study has confirmed that an association exists between the use of oxytocin in labour and neonatal hyperbilirubinaemia, and has eliminated low birthweight, duration of labour and method of feeding as significant aetiological factors.

Further investigation will be necessary to determine the true nature of the association and work is currently in progress to study the frequency of hyperbilirubinaemia when labour has been induced with prostaglandins.

Meanwhile, the immense value of oxytocin in the induction and acceleration of labour is beyond question and on present evidence there is no reason to suppose that the degree of bilirubinaemia which follows the use of oxytocin to induce labour is harmful to the newborn. It would seem prudent, however, to consider an alternative method of stimulating labour in mothers whose babies run the risk of developing neonatal hyperbilirubinaemia because of conditions such as rhesus isoimmunization or extreme immaturity.

## ACKNOWLEDGEMENTS

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# AN EVALUATION OF HUMAN PLACENTAL LACTOGEN LEVELS IN HYPERTENSION OF PREGNANCY

BY

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## Summary

Maternal plasma levels of human placental lactogen (HPL) are decreased in hypertensive pregnancies and the concentration is related to the severity of the condition. Of infants who had HPL concentrations in the fetal danger zone (more than 2 SD's below normal mean) 75 per cent developed fetal distress, neonatal asphyxia or were light for dates. Patients who are identified in this way have lighter infants and placentae than their unidentified counterparts with normal HPL levels.

HYPERTENSION is a well recognized complication of pregnancy and associated with an increased perinatal mortality and morbidity (Butler and Bonham, 1963). The early work of Kaplan and Grumbach (1964) and Sciarra *et al.* (1963) which identified the placenta as the sole organ to produce HPL, directed much attention to its role as an indicator of placental function. In 1971, Spellacy *et al.* (1971) and Keller *et al.* (1971), in independent studies on hypertensive patients, described excellent results when using HPL to predict fetal mortality but they were not supported by Letchworth and Chard (1972) whose results were inconclusive.

## PATIENTS AND METHODS

The patients investigated were 308 with hypertension developing after the 28th week of pregnancy. They were divided into three groups depending on the highest diastolic blood pressure attained on two consecutive occasions. The 175 patients in the mild group had a reading of 90 to 99 mm. Hg, the 95 patients in the moderate group 100 to 109 mm. Hg and the 37 patients in the severe group had pressures greater than 110 mm. Hg, or more than 90 mm. Hg with proteinuria of at least 1·0 g./24 hours. A normal group of 429 patients has already been described by England *et al.* (1974), who also defined our criteria for fetal distress and neonatal asphyxia. A baby was considered to be light for dates when its birthweight was below the tenth percentile for the period of gestation (Battaglia and Lubchenco, 1967). Blood specimens were taken in most cases on more than one occasion after 32 weeks for HPL, urea and creatinine and 24 hour urines were collected for creatinine and protein determinations. The specimens for HPL were stored at −10 °C. prior to analysis and other investigations were performed within four hours. Plasma HPL was estimated by radioimmunoassay as previously described (England *et al.*, 1974). Urea and creatinine were quantitated on the Auto Technicon II analyzers by the A.AII-01 and A.AII-11 methods respectively. Proteins in urine were precipitated with trichloracetic acid prior to quantitation with biuret reagent.

## RESULTS

Plasma HPL concentrations of normal and hypertensive patients from 32 weeks gestation appear in Table I. There is considerable overlap between the four groups and in comparing levels in the normal and mild hypertension groups the difference is only statistically significant at 34 to 36 weeks (p<0·01, 0·001 and 0·05 respectively) and at 40 weeks (p<0·005) whereas in comparing the normal and severe hypertension groups the difference is significant from 32 to 35 weeks (p<0·001, 0·01, 0·005 and 0·001 respectively). The difference between the mild and moderate hypertension groups is not significant.

In 308 pregnancies associated with hypertension, 104 babies developed fetal distress, neonatal asphyxia and/or were light for dates; four were stillborn. In the mildly hypertensive group there were 2 stillbirths and 58 whose condition was unsatisfactory during the neonatal period; in the moderately hypertensive group, one stillbirth and 31 affected babies and the severely hypertensive group 15 affected babies and one stillbirth. In Table II the HPL concentrations of all hypertensive patients whose infants were normal are compared to the group in whom neonatal complications arose. Apart from the 32nd gestational week there is a marked difference between the groups.

HPL concentrations which were 2 SD below the normal mean for the period of gestation were said to be in the fetal danger zone (FDZ) and were considered to indicate a fetus in jeopardy even when occurring on only one occasion. Based on this criterion 46 of the 108 affected babies were successfully predicted—19 in the mild, 17 in the moderate, and 10 in the severe groups. Four of the remaining 62 babies had the cord tightly round the neck at birth and had fetal distress which was not indicated by low HPL concentrations. In the mildly and moderately hypertensive groups 15 mothers who had normal infants had low HPL levels on 18 occasions but the level returned to normal prior to delivery.

Light for dates infants were associated with repeatedly low HPL levels in 70·0 per cent of cases and levels which fluctuated between normal and FDZ in 30·0 per cent. In 30·0 per cent of the asphyxiated group continually low levels were found and a further 5·0 per cent were on occasion in the FDZ. In 34·5 per cent of cases with fetal distress there were variable HPL levels which did not remain in the FDZ unless associated with a light for dates infant or asphyxia as shown in Table III.

Reduction of glomerular filtration could give

TABLE I

*HPL concentrations in normal and hypertensive pregnancies. The number of specimens in each subgroup is given in parentheses*

| | HPL concentrations (mg./l.) Mean±SD | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 32 weeks | 33 weeks | 34 weeks | 35 weeks | 36 weeks | 37 weeks | 38 weeks | 39 weeks | 40 weeks |
| Normal | 4·4±0·8<br>(32) | 4·6±1·0<br>(12) | 4·9±0·9<br>(27) | 5·5±1·0<br>(50) | 5·3±1·0<br>(63) | 5·1±1·0<br>(40) | 5·3±0·9<br>(52) | 5·2±1·1<br>(28) | 5·5±0·9<br>(40) |
| Mild hypertension | 4·3±1·3<br>(20) | 5·0±1·4<br>(12) | 4·2±1·1<br>(24) | 4·5±1·3<br>(45) | 4·8±1·6<br>(49) | 5·1±1·6<br>(48) | 5·0±1·5<br>(67) | 4·7±1·4<br>(94) | 4·6±1·4<br>(53) |
| Moderate hypertension | 4·6±1·6<br>(9) | 4·5±1·6<br>(8) | 4·9±1·6<br>(9) | 4·5±1·1<br>(24) | 5·0±1·3<br>(36) | 4·8±1·3<br>(28) | 5·0±1·1<br>(54) | 4·9±1·5<br>(43) | 5·0±1·4<br>(32) |
| Severe hypertension | 3·2±1·6<br>(15) | 3·2±1·2<br>(10) | 3·6±1·6<br>(15) | 4·2±1·5<br>(24) | 4·7±1·5<br>(18) | 4·4±1·3<br>(17) | 5·1±1·7<br>(20) | 4·8±1·7<br>(22) | — |

TABLE II

*HPL concentration in hypertensive pregnancies with relation to fetal outcome. The number of specimens in each subgroup is given in parentheses*

| | HPL concentration (mg./l.) Mean±SD | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 32 weeks | 33 weeks | 34 weeks | 35 weeks | 36 weeks | 37 weeks | 38 weeks | 39 weeks | 40 weeks |
| Normal baby | 4·7±1·6<br>(14) | 4·9±1·1<br>(11) | 4·5±1·2<br>(23) | 4·9±1·0<br>(31) | 5·4±1·3<br>(40) | 5·1±1·0<br>(35) | 5·2±1·1<br>(57) | 5·3±1·3<br>(55) | 5·3±1·3<br>(38) |
| Fetal distress, neonatal asphyxia, light-for-dates or stillbirth | 3·7±1·6<br>(22) | 3·7±1·6<br>(17) | 3·0±1·4<br>(18) | 3·7±1·5<br>(31) | 4·0±1·5<br>(35) | 4·1±1·6<br>(32) | 4·4±1·6<br>(35) | 4·2±1·3<br>(51) | 4·2±1·4<br>(40) |
| Significance of difference | p<0·10 | p<0·05 | p<0·005 | p<0·001 | p<0·001 | p<0·005 | p<0·01 | p<0·001 | p<0·005 |

TABLE III

*Relationship between HPL levels and condition of fetus*

| Fetal condition | Number of patients | | Correct predictions (per cent) |
|---|---|---|---|
| | Normal HPL levels | Low HPL levels | |
| Normal | 185 | 15 | 92·5 |
| Fetal distress | 19 | 10 | 34·5 |
| Neonatal asphyxia | 24 | 13 | 35·0 |
| Fetal distress and neonatal asphyxia | 9 | 6 | 40·0 |
| Light-for-dates | 0 | 6 | 100·0 |
| Light-for-dates and neonatal asphyxia and fetal distress | 4 | 9 | 69·0 |

rise to falsely elevated HPL levels and thus explain the 47 per cent detection rate for babies in jeopardy. Table IV shows plasma urea and creatinine clearance values after 35 weeks gestation for the mothers of affected infants who had HPL levels in the FDZ and for those who had normal HPL concentrations. There was no significant difference between the two groups.

The relationship of placental weight, after 35 weeks gestation, to increasing blood pressure was investigated. In the normal blood pressure group the mean weight was 665 g. (SD 130), in the mildly hypertensive group 667 g. (SD 138), with moderate hypertension 636 g. (SD 145) and in the severely hypertensive group 626 g. (SD 182) demonstrating no difference between the groups. However, when the placental weight of distressed fetuses and neonatally asphyxiated babies associated with low and normal HPL concentrations were compared there was a marked difference in the hypertensive groups but not in the normal group. Similarly, when the weights of babies of at least 38 weeks gestation whose condition had been predicted by low maternal HPL levels were compared with those who had normal maternal HPL levels, there was again a significant difference in the hypertensive groups but not in the normal group. These results appear in Table V.

## DISCUSSION

Investigators have described high, normal and low HPL levels in hypertensive pregnancies (see Letchworth *et al.*, 1972), but many of the studies were limited to a small number of patients. Keller *et al.* (1971), Spellacy *et al.* (1971), Letchworth *et al.* (1972) and Lindbergh and Nilsson (1973) tested larger populations and all reported low values with which our findings agree. Spellacy *et al.* (1971) has also described decreasing HPL concentrations with increasing hypertension. We were able to confirm these

TABLE IV

*Plasma urea and creatinine clearances in hypertensive pregnancies. The number of specimens in each subgroup is given in parentheses*

| | Mild hypertension | Moderate hypertension | Severe hypertension | Low HPL and complications | Normal HPL and complications |
|---|---|---|---|---|---|
| Plasma urea (mg./100 ml.) Mean ±SD | 18±5<br>(211) | 21±6<br>(131) | 21±7<br>(149) | 22±8<br>(37) | 21±11<br>(35) |
| Creatinine clearance (ml./minute) Mean±SD | 110±33<br>(93) | 99±23<br>(53) | 103±31<br>(52) | 113±14<br>(10) | 109±37<br>(14) |

TABLE V

*Birthweight and placental weight in normal and hypertensive pregnancies associated with fetal distress and neonatal asphyxia. The number of patients in each subgroup is given in parentheses*

| | Birth weight (kg.) Mean±SD | Placental weight (g.) Mean±SD |
|---|---|---|
| Hypertension and low HPL level | 2·95±0·60<br>(34) | 567±187<br>(34) |
| Hypertension and normal HPL level | 3·33±0·59<br>(59) | 671±157<br>(50) |
| Significance of difference | p<0·005 | p<0·01 |
| Normal and low HPL | 3·09±0·61<br>(28) | 598±140<br>(23) |
| Normal and normal HPL | 3·24±0·49<br>(45) | 660±157<br>(43) |
| Significance of difference | N.S. | N.S. |

N.S. = Not significant.

results and therefore did not find that mild hypertension was associated with lower maternal HPL levels than the severe hypertension as described by Letchworth *et al.* (1972).

Low HPL levels were found by Letchworth *et al.* (1972) to be associated with fetal complications only in the multipara and they suggested that one could not differentiate between low values due to the maternal hypertensive condition or to the fetus "at risk". Taking no account of parity we found significantly lower HPL concentrations associated with fetal complications and when it was in the FDZ, 75 per cent developed fetal distress, neonatal asphyxia and/or were light for dates. Although we excluded infants with the cord round the neck, 21 per cent of patients with normal levels also developed fetal complications. In this study three stillbirths and one neonatal death occurred. One stillbirth with no congenital abnormalities had low maternal HPL levels. Normal maternal HPL levels were found in the second stillbirth and in the neonatal death, both with congenital abnormalities and in the third stillbirth associated with abruptio placentae. However, in these three cases, one would not have expected a reduction of placental function. Spellacy *et al.* (1971) assessed the ability of HPL to predict only fetal mortality and with an incidence of 7 per cent in his population he found all to have low HPL levels. Our group has a 1·3 per cent perinatal mortality and due to the small number of fetal deaths and the pathological findings no valid comparison can be made.

The 55 per cent failure in the detection rate suggests that plasma HPL concentration does not always reflect the production rate. As HPL has no diurnal variation (Lindbergh *et al.*, 1972), and is thought to be unaffected by uterine blood flow (Niven and Spellacy, 1974) and by the patient's activity (Letchworth *et al.*, 1974), it was considered that a reduction of glomerular filtration could lead to a falsely normal plasma level. Nachtigall *et al.* (1968) and Carrington *et al.* (1970) have studied oestriol excretion in toxaemia and found it to be dependent on renal function. Hypertension is associated with low creatinine clearances and elevated plasma ureas, the latter being proportional to the severity of the condition (Bucht and Werko, 1953). However, we were unable to demonstrate any statistically significant difference between the three groups. When renal function of patients whose infants had complications in the neonatal period were compared, no difference was found between those in whom the conditions had been predicted by low maternal HPL levels and those with normal levels.

Spellacy *et al.* (1966) found no correlation between placental weight and maternal plasma HPL concentration and although others have found a positive correlation it is too low to be of predictive value (Sciarra *et al.*, 1968). Genazzini *et al.* (1971) has observed that HPL levels fall in labour, and suggests that this is due to uterine contraction decreasing placental blood flow and that a closer correlation is observed when the hormone is estimated prior to labour. Ursell *et al.* (1973) when investigating diabetic pregnancies suggested that elevated HPL levels reflected the increased placental mass. Chard (1973) has suggested that severe toxaemia is associated with large placentae but our figures did not support this view, no difference being found between the three groups, although in the severe group the range was greater. We did find, however, that in patients where the maternal HPL values correctly predicted fetal complications the placentae were smaller than in the 55 per cent of whom a normal fetal state was incorrectly forecast. This difference was not found in normal pregnancies with fetal complications and may suggest that an equivalent weight hypertensive placenta has less metabolic reserve and will not sustain the fetus through the stresses of labour.

Maternal HPL concentration and fetal weights have been studied by Spellacy *et al.* (1966), Sciarra *et al.* (1968) and Letchworth *et al.* (1971) in normal and clinically undefined pregnancies and no correlation found. However, Cramer *et al.* (1971) did show a relationship after allowing for gestation and in diabetic pregnancies Ursell *et al.* (1973) described a correlation in this condition associated with large infants. Fetal weight probably reflects placental weight. It is therefore not surprising that there is a highly significant difference in the hypertensive but not the normal group regarding the weights of infants whose fetal complications were predicted by HPL levels in the FDZ and those for whom the test failed.

During this investigation it became apparent that certain complications in the perinatal period were detected with varying degrees of success and were associated with different HPL patterns. The light-for-dates infant was the most frequently predicted and together with the neonatally asphyxiated baby was distinguished by HPL levels repeatedly in the FDZ. Fetal distress, when identified, was indicated by oscillating levels.

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# SERUM CYSTINE AMINOPEPTIDASE AND THE NORMAL WEIGHT BABY IN NORMOTENSIVE AND HYPERTENSIVE PREGNANCY

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## Summary

The levels of serum cystine aminopeptidase have been studied serially in normotensive and hypertensive mothers who delivered healthy, normal weight-for-dates babies. A normal range, both for enzyme level and maximum and minimum rates of increase of the enzyme during gestation, was established for each group, those of the hypertensive patients exhibiting different characteristics from the curves in normotensive patients. It is suggested that such differences be considered when assessing pregnancies complicated by hypertension.

A NUMBER of workers have suggested that levels of serum cystine aminopeptidase (CAP) in pregnancy might be employed to some advantage as an index of placental function (Babuna and Yenen, 1966; Josephides and Turkington, 1967; Tovey, 1969). We have studied the concentration of this enzyme in maternal serum in normal and "at risk" pregnancies, assaying CAP levels serially and being particularly concerned with the position of the small-for-dates infant. In our first series of patients we selected normal subjects at the booking clinic according to criteria which took account of past history and the obstetric state of the patient at the time of booking, with a postnatal assessment which resulted in the exclusion of a number of patients originally included as normal (Chapman *et al.*, 1971).

A primary necessity in our studies was the establishment of a normal range for the enzyme in pregnancy using the method of Roth (1964). This was obtained by fitting a regression line to the logarithm of the CAP readings and calculating the 95 per cent confidence limits of the line in the usual way. We pointed out that the statistical assumptions underlying the derivation of the normal range were not strictly accurate; they resulted in a curve which gave higher values after 250 days than those actually observed. We suggested, therefore, that this range, while of use for first working purposes, should only be regarded as providing preliminary values. Subsequently, we published a revised normal range (Chapman *et al.*, 1973) which takes account of the essential S-shaped nature of normal CAP curves, i.e. after 250 days of gestation the CAP level rises at a decreasing rate.

At the same time, we also advanced the suggestion that the actual values of the enzyme, whether inside or outside the limits of the normal range, yielded only part of the information held in the CAP curves. Of equal or possibly greater importance was consideration of the rate of increase or decrease in the CAP level, particularly over short periods of a few weeks during gestation. We therefore introduced the idea of maximum and minimum limits or gradients for the normal rate of increase of CAP over short periods. In any one patient, all the CAP readings obtained could fall within the limits of the normal range, but if the slope of a line drawn through the points over a short period of a few weeks did not compare with the suggested normal gradient for that period, then such a patient could not be considered as normal.

One of the "at risk" groups studied were those patients whose pregnancy was complicated by hypertension, a condition which may dispose to intrauterine retardation of fetal growth. Certain interesting features emerged from the examination of the data observed in these patients, in particular those hypertensive patients who delivered babies which were of normal weight for gestational age. These findings were evaluated by comparing them with the data obtained in normal pregnancies. The ultimate aim in establishing a normal range and normal gradients for CAP was to be able to use them to predict abnormality. However, we considered that it would be inadvisable to use these limits in the case of hypertensive mothers delivering normal weight for-dates infants, since, in general, we had observed that the CAP curves demonstrated by these mothers were appreciably different from the curves in normal pregnancies. Consequently, we decided to calculate separate "normal" ranges and gradients for normotensive and hypertensive mothers.

## SELECTION OF PATIENTS

### *Population sample*

Our group of hospitals maintains an obstetric unit which serves a population of some 250 000, and delivers 3000 women per annum. Of these, approximately 10 per cent are non-European.

### *Patients*

*Normotensive patients.* Patients were considered to be normal or normotensive if the pregnancy followed a normal course with the delivery of a healthy infant of normal birthweight whose neonatal period was uneventful. Normal birthweight was defined as being above the 10th centile (Thomson *et al.*, 1968). The data from 170 normotensive patients was assessed. Between 3 and 25 CAP readings were obtained from each patient, the average number being about 10.

*Hypertensive patients.* The data were evaluated from 123 patients suffering from hypertension, defined as a blood pressure level in excess of 140/90 mm. Hg; all delivered a healthy infant of normal birthweight with a normal neonatal period. We excluded diabetic patients who developed a hypertensive state. The number of CAP readings per patient was similar to those of the normal patients.

## METHODS AND RESULTS

### *Assay of serum cystine aminopeptidase (CAP)*

When possible, sera were assayed on the day of collection. Otherwise, samples were stored at −20 °C. and assayed within 48 hours by an automated adaptation of Roth's manual spectrofluorimetric method (Roth, 1964; Chapman *et al.*, 1972). This method depends on the release of β-naphthylamine from a fluorogenic substrate, L-cystine-di-β-naphthylamide, when pregnancy serum is added to the incubation mixture. The intensity of fluorescence is proportional to the amount of β-naphthylamine released and in turn to the concentration of the enzyme in the serum. Results are expressed in terms of automated units rather than as IU/l, as there is some controversy as to whether one or two molecules of β-naphthylamine are released during the enzymic hydrolysis (Klimek, 1968).

### *Calculation of the CAP range in normotensive and hypertensive pregnancy*

Because the CAP curves in normotensive patients do not conform to a simple pattern, none of the usual methods for fitting confidence limits could be used successfully. The statistical approach finally adopted was empirical to some extent and similar to that used previously (Chapman *et al.*, 1973). It was applied to the data obtained from normotensive patients, and hypertensive patients who delivered an infant of normal birthweight.

Table I shows the distribution of 566 and 421 readings for normotensive and hypertensive women respectively. We plotted the CAP readings obtained from both groups of patients and then instead of considering the period of

TABLE I

*Distribution of observations between time intervals used for calculation of 95 per cent confidence intervals for CAP in normotensive and hypertensive patients with normal weight babies*

| Weeks | Normotensive | Hypertensive |
|---|---|---|
| 20–23 | 49 | 28 |
| 24–27 | 78 | 34 |
| 28–29 | 59 | 41 |
| 30–31 | 63 | 52 |
| 32–33 | 61 | 56 |
| 34–35 | 63 | 65 |
| 36–37 | 69 | 70 |
| 38–39 | 64 | 60 |
| 40–41 | 60 | 15 |

TABLE II

*The 95 per cent confidence intervals for the normal range of CAP in automated units in normotensive and hypertensive patients giving birth to normal weight babies*

| Weeks of gestation | Normotensive patients | | Hypertensive patients | |
|---|---|---|---|---|
| | Lower limit | Upper limit | Lower limit | Upper limit |
| 20 | 9·5 | 23·5 | 9·5 | 24·0 |
| 21 | 9·5 | 24·5 | 9·5 | 26·0 |
| 22 | 9·5 | 25·5 | 9·5 | 28·0 |
| 23 | 10·0 | 27·0 | 10·0 | 30·0 |
| 24 | 10·0 | 29·0 | 10·0 | 32·0 |
| 25 | 10·5 | 31·0 | 10·5 | 34·5 |
| 26 | 11·0 | 33·5 | 11·5 | 36·5 |
| 27 | 12·0 | 36·5 | 12·5 | 39·5 |
| 28 | 12·5 | 39·5 | 14 0 | 42·0 |
| 29 | 13·5 | 44·0 | 15·5 | 46·5 |
| 30 | 15·0 | 47·5 | 17·0 | 51·5 |
| 31 | 17·0 | 51·5 | 18·5 | 57·5 |
| 32 | 19·0 | 57·0 | 21·0 | 64·5 |
| 33 | 20·5 | 62·5 | 23·5 | 74·0 |
| 34 | 22·0 | 68·0 | 26·5 | 83·5 |
| 35 | 24·0 | 73·5 | 30·0 | 90·0 |
| 36 | 25·5 | 78·5 | 32·0 | 95·5 |
| 37 | 28·0 | 84·0 | 33·0 | 100·5 |
| 38 | 31·0 | 90·0 | 32·5 | 103·0 |
| 39 | 32·0 | 94·0 | 32·0 | 105·0 |
| 40 | 32·0 | 97·0 | 31·5 | 105·0 |
| 41 | 31·0 | 99·0 | 31·5 | 105·0 |

gestation as a whole, we divided it into nine time periods, so that the "S" portion of the curve (Fig. 1) could be treated more appropriately. The time periods were from 20 to 23, 24 to 27, 28 to 29, 30 to 31, 32 to 33, 34 to 35, 36 to 37, 38 to 39 and 40 to 41 weeks. Not more than one reading was used from any patient in any time period and only three or four readings were used from any one patient throughout; the readings were chosen randomly.

Linear regression lines were fitted to the $\log_e$ CAP for each time period and the corresponding 95 per cent confidence limits calculated for each period. Finally, lines were drawn joining those points of the confidence limits that corresponded to the central day of each period. The resulting overall 95 per cent confidence limits thus obtained for each group of patients was used as their normal range respectively. The 95 per cent confidence limits for the two groups of patients for each week of pregnancy from 20 to 41 weeks are given in Table II.

*Calculation of maximum and minimum rates of increase of CAP*

The time between the 24th and 40th week of gestation was divided into four-week periods. This allowed for the possibility of hypertensive patients delivering before term and for the current practice of planned induction. In order to obtain a good estimate of a slope over a four-week period, it was appreciated that sufficient readings spanning that period would have to be available. The following conditions of entry were therefore applied: for a patient to be allocated to any one of the four-week periods, there had to be four CAP readings, the first within one week of the beginning of the period and the last reading within one week of the end of the period.

Simple linear regression lines were then fitted to the selected points from each patient for each time period. Using the slopes thus obtained, confidence intervals were then calculated for the rate of increase or decrease of CAP for each period.

*Hypertensive patients with normal weight babies*

During the period 24 to 28 weeks, we did not obtain sufficient readings for the calculation of confidence intervals. Consequently, only limits for the remaining three intervals have been calculated. There were 27 patients in each of these three groups. The distribution of the slopes

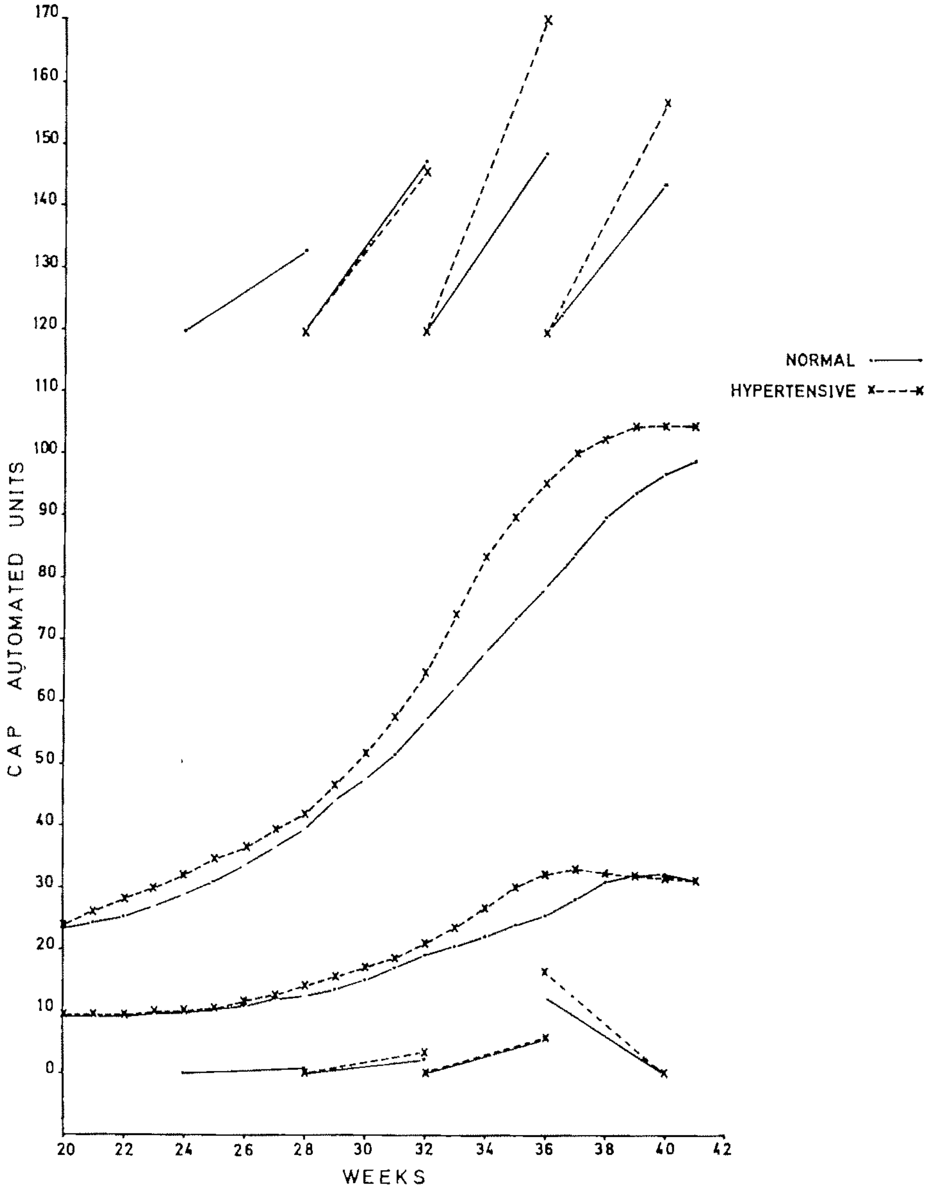

FIG. 1

The normal range of serum CAP and corresponding maximum and minimum gradients in normotensive and hypertensive patients with normal weight infants.

differed in each of the time periods as Figure 2 shows. In order to avoid assumptions about the precise distributions in these three periods, approximate confidence intervals at the 95 per cent, 90 per cent and 80 per cent level were estimated by inspection, such that not more than 5 per cent, 10 per cent and 20 per cent of the slopes respectively fell outside the intervals. The levels are shown in Table III. There were two main considerations in deciding on the limits of

TABLE III

*Non-calculated confidence intervals at varying levels of maximum and minimum gradients for CAP in automated units per day in hypertensive patients with normal weight babies*

| Approximate confidence interval | Weeks of gestation | | | | | |
|---|---|---|---|---|---|---|
| | 28–32 | | 32–36 | | 36–40 | |
| | Minimum | Maximum | Minimum | Maximum | Minimum | Maximum |
| 95 per cent | 0·09 | 0·94 | 0·18 | 1·95 | —0·77 | 2·01 |
| 90 per cent | 0·11 | 0·94 | 0·19 | 1·81 | —0·59 | 1·34 |
| 80 per cent | 0·14 | 0·88 | 0·21 | 1·64 | —0·48 | 1·25 |

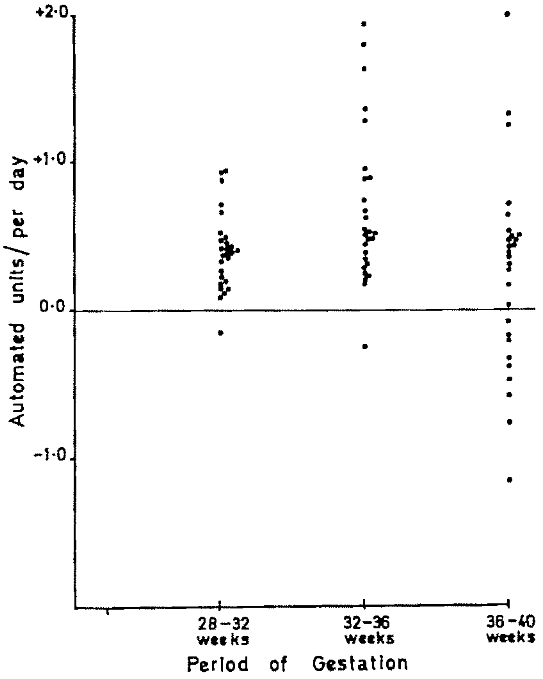

FIG. 2

Rates of increase of serum CAP (as automated units per day) in hypertensive patients with normal weight infants over separate four-week periods.

any interval. If the number of the slopes to be excluded from the interval was even, then the limits were chosen so that exactly half the excluded slopes were below the lower band and half were above the upper band. If the number of slopes to be excluded from the interval was odd, then the extra slope to be excluded was chosen so as to make the confidence interval as narrow as possible. For practical purposes, the use of the 90 per cent confidence interval is recommended and the resulting maximum and minimum limits (or gradients) are shown in Figure 1.

*Normotensive patients*

Apart from the 24 to 28 week period which contained data on 31 patients, each of the other three periods contained data on 58 patients. Again, Figure 3 shows that the distribution of

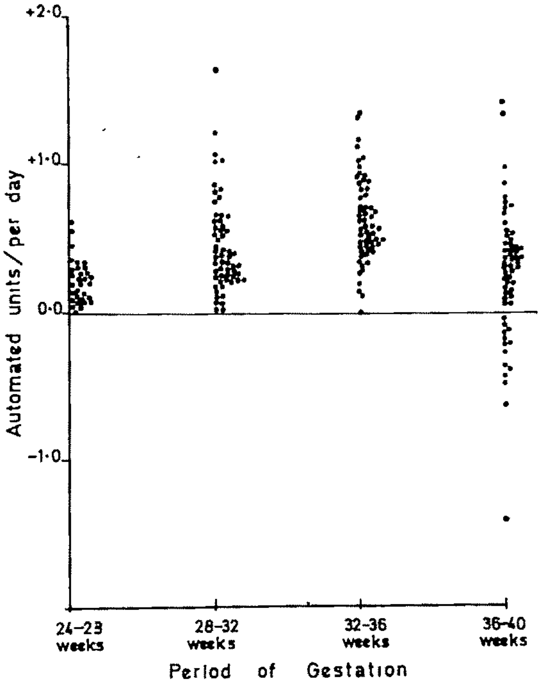

FIG. 3

Rates of increase of serum CAP (as automated units per day) in normotensive patients over separate four-week periods.

TABLE IV

*Non-calculated confidence intervals at varying levels of maximum and minimum gradients for CAP in automated units per day in normotensive patients with normal weight babies*

| Approximate confidence interval | Weeks of gestation | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 24–28 | | 28–32 | | 32–36 | | 36–40 | |
| | Minimum | Maximum | Minimum | Maximum | Minimum | Maximum | Minimum | Maximum |
| 95 per cent | 0·01 | 0·57 | 0·02 | 1·07 | 0·12 | 1·18 | −0·64 | 0·98 |
| 90 per cent | 0·03 | 0·47 | 0·08 | 1·00 | 0·19 | 1·12 | −0·44 | 0·87 |
| 80 per cent | 0·08 | 0·37 | 0·16 | 0·83 | 0·32 | 0·97 | −0·28 | 0·72 |

the slopes differed in each of the time periods and again assumptions about the precise distributions were avoided. As in the case of the hypertensive patients, approximate confidence intervals were estimated at varying levels shown in Table IV, the 90 per cent level again being recommended for practical purposes. The gradients are shown in Figure 1.

## DISCUSSION

During studies of serum CAP as an index of placental function in normal and "at risk" pregnancies, we became aware that in addition to the normal range established from data derived from normal pregnancies, there might also be a "normal" or acceptable range in hypertensive patients who delivered normal weight infants. This "normal range" might subsequently find considerable use in the evaluation of CAP curves in pregnancies complicated by hypertension, more especially those where the ultimate birth of a small-for-dates baby could be expected. There have been indications in the results of other workers that this could be the case (Tovey, 1969; de Bruijckere, 1970; Ryden, 1972). Conversely, Petrucco *et al.* (1973) stated that they were unable to detect any difference in the mean CAP values and rate of increase of CAP values in normotensive and hypertensive patients delivered of normal infants.

Applying the statistical treatment described above to our data, derived by an automated technique, on normotensive and hypertensive pregnant patients delivering babies which were healthy and normal in weight for gestational age, a normal range was found for each group respectively and two corresponding sets of maximum and minimum allowable gradients for the increase or decrease of CAP over short periods of gestation. It will be seen from Figure 1 that the "normal" or acceptable range in hypertension is above the range in normotensive patients, the upper limit inclining more steeply after 30 weeks, but tailing off rather earlier at 36 to 37 weeks. The lower limit also rises more steeply than the normotensive range after 33 weeks and tends to fall after 37 weeks, finally coinciding with the normotensive range.

The levels of CAP demonstrated by the curve of the hypertensive patient appear to be about four weeks in advance of those shown by the normotensive patient, thus the level of CAP in general is that much higher in hypertensive patients. Similarly, the plateau parts of the hypertensive curve, both lower and upper limits, are reached about one month before those of the normal curve. The maximum and minimum gradients are also affected as can be seen from Figure 1, particularly the maximum gradients from 32 weeks and the minimum gradient from 36 weeks.

It is our submission that, using the confidence intervals given above, one can predict reasonably well a small-for-dates baby if the CAP gradients and/or levels are outside the relevant limits, both in the case of the normotensive and hypertensive mother.

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# BETA-MIMETIC DRUGS AND POSSIBLE PREVENTION OF RESPIRATORY DISTRESS SYNDROME

BY

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## Summary

The prophylactic value of beta-mimetic drugs in preventing respiratory distress syndrome (RDS) is investigated by comparing 29 preterm infants of ritodrine treated mothers with 34 preterm infants of a control group. Five cases of RDS (17 per cent) occurred among the infants of treated mothers as compared with 12 cases (35 per cent) in the control group ($p = 0 \cdot 09$). The difference is, however, statistically significant for the group of infants weighing less than 2300 grams ($p = 0 \cdot 01$) which includes all infants affected with RDS.

A number of drugs have been reported to stimulate lung maturation (Glass *et al.*, 1971; Redding and Douglas, 1972; Liggins and Howie, 1972), but only one well-controlled clinical trial has demonstrated a significant effect of any maternal medication in the prophylaxis of respiratory distress syndrome (RDS) in premature newborn babies (Liggins and Howie, 1972).

Recently Kero *et al.* (1973) proposed that maternal administration of isoxsuprine, a beta receptor stimulant with weak alphalytic activity, was associated with a reduced incidence of respiratory distress syndrome (RDS) in premature babies. Liu (1973) proposed a similar effect of a methylxanthine and suggested that the effect of isoxsuprine and the methylxanthine was to increase adrenal cortical steroidogenesis and as a consequence stimulate lung maturation.

Neither of the two authors cited data from control groups. Since 1970 we have been investigating in this clinic a beta-mimetic agent, ritodrine, for the treatment of premature labour. Although it is a very effective uterine relaxant there still are occasions when it is not possible to prolong pregnancy until term.

In 1972 we noticed that the incidence of RDS in the preterm liveborn babies whose mothers had received ritodrine appeared to be surprisingly low. To investigate this possibility further we retrospectively compared the incidence of RDS in the preterm babies of mothers treated with ritodrine with the incidence of RDS in the preterm infants born at the same clinic but whose mothers had not received any beta-mimetic drug.

## METHODS

Included in this survey were the 29 liveborn premature infants born of ritodrine-treated mothers during the years 1970, 1971, 1972 and part of 1973, and the 34 liveborn premature infants born in the same clinic of mothers who had not received ritodrine.

Excluded from both groups in the study were all the patients with premature rupture of

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membranes, with fetal malnutrition, malformations and pathological presentations. All infants were observed and treated in the same neonatal care unit and at that time neither paediatricians nor obstetricians were aware that a comparison of this nature would be made.

Respiratory distress syndrome was diagnosed on the basis of respiratory rate, grunting, chest retraction and radiological signs.

A comparison was made between the two groups of the incidence of RDS related to duration of gestation at birth based upon the reported duration of amenorrhoea and the incidence of RDS related to birthweight.

## RESULTS

Five cases of RDS (17 per cent) occurred among the infants of ritodrine-treated mothers as compared with 12 cases (35 per cent) in the control group.

The chi-square test of the difference in favour of ritodrine yields a one-sided p-value of 0·09 and thus provides some support for the initial suggestion that ritodrine reduces the incidence of RDS. A more sensitive test of the difference between the ritodrine-treated and control groups is obtained by confining the comparison to those patients whose babies were most prone to develop RDS in that they were short of gestational age and low birthweight. Of these two parameters, birthweight is the more important and generally the more reliable. The total range of birthweights in this study was between 900 and 2700 g. All cases of RDS occurred at birthweights below 2300, as may be seen from Table I.

Application of the chi-square test to the first three lines of the Table yields a one-sided p-value equal to 0·01 in favour of ritodrine (two-sided p = 0·02). Details of the individual patients in the first three lines of Table I are given in Table II. Table II gives an impression of the comparability of the two groups, as far as birthweights less than 2300 g. are concerned.

The most notable feature is the small number of male infants in the control group, compared to the ritodrine-treated group. Since the only available evidence of a difference between sexes with respect to incidence of RDS indicates that males may be somewhat more vulnerable (Taylor, 1971), this difference between the two groups if anything, strengthens the conclusion of the previous paragraph. Figure 1 shows the incidence of RDS in the two groups by birthweight.

TABLE I

*The incidence of respiratory distress syndrome (RDS) in control infants and those whose mothers had been treated with ritodrine, in two birthweight groups*

| | | Ritodrine | Control |
|---|---|---|---|
| Birthweight less than 2300 g. | RDS | 5 | 12 |
| | No RDS | 15 | 6 |
| Total number weighing less than 2300 g. | | 20 | 18 |
| Birthweight 2300 g. or more | RDS | 0 | 0 |
| | No RDS | 9 | 16 |
| Total number weighing 2300 g. or more | | 9 | 16 |
| Total | | 29 | 34 |

Four infants in the ritodrine-treated group died in the neonatal period, two of RDS, one of septicaemia, and one of listeriosis. The intervals between onset of treatment and delivery were 90 minutes and 57 hours for the two infants

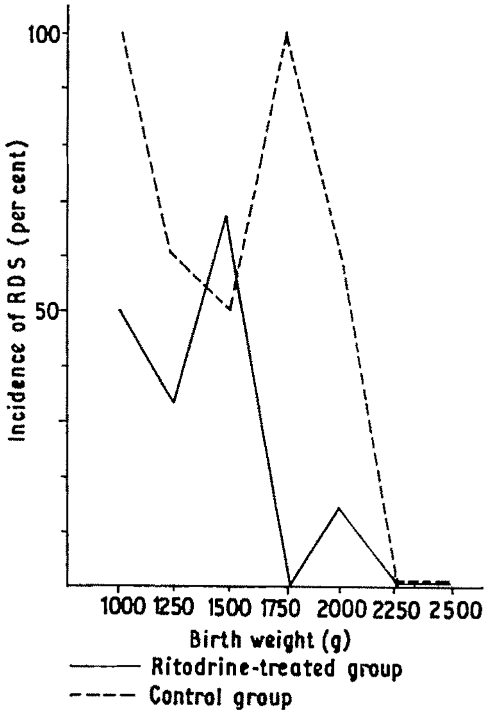

FIG 1

TABLE II
*Details of infants with a birthweight of less than 2300 g.*

| | Gestational age (weeks) | Birthweight (g.) | Sex | Apgar score (1 minute) | Outcome |
|---|---|---|---|---|---|
| Ritodrine-treated mothers, RDS | 25 | 900 | M | 5 | Died |
| | 30 | 1500 | F | 4 | Died (septicaemia) |
| | 30 | 1600 | M | 2 | Died (listeriosis) |
| | 33 | 1600 | M | 7 | Died |
| | 36 | 2200 | M | 9 | Survived |
| Ritodrine-treated mothers, no RDS | 28 | 1200 | F | 10 | Survived |
| | 29 | 1400 | M | 9 | Survived |
| | 31 | 1300 | M | 6 | Survived |
| | 33 | 1600 | M | 6 | Survived |
| | 31 | 1800 | M | 8 | Survived |
| | 33 | 1800 | M | 7 | Survived |
| | 34 | 1800 | M | 8 | Survived |
| | 34 | 1800 | F | 5 | Survived |
| | 33 | 1900 | M | 10 | Survived |
| | 34 | 2100 | F | 4 | Survived |
| | 35 | 2100 | F | 8 | Survived |
| | 36 | 2100 | F | 3 | Survived |
| | 36 | 2100 | M | 9 | Survived |
| | 35 | 2200 | M | 2 | Survived |
| | 36 | 2250 | F | 10 | Survived |
| Controls, RDS | 25 | 900 | M | 6 | Died |
| | 26 | 900 | F | 6 | Died |
| | 29 | 1250 | F | 5 | Survived |
| | 29 | 1300 | M | 10 | Died |
| | 31 | 1350 | F | 7 | Died |
| | 31 | 1400 | M | 6 | Died |
| | 31 | 1600 | M | 10 | Died |
| | 32 | 1600 | F | 10 | Died |
| | 35 | 2000 | F | 10 | Survived |
| | 35 | 2000 | F | 4 | Survived |
| | 34 | 2200 | F | 7 | Survived |
| | 36 | 2250 | F | 4 | Survived |

who died of RDS. Eight control infants died, seven from RDS and one from pulmonary haemorrhage.

The development of the surviving infants was satisfactory as assessed by examination at six weeks of age.

## DISCUSSION

The results of this retrospective study tend to support the suggestions of Kero *et al.* (1973) and Liu (1973) with isoxsuprine and methylxanthine.

For the group of babies weighing less than 2300 g., which included all the babies affected with RDS, the incidence of RDS was significantly lower after maternal treatment with ritodrine than in the control group. It has to be emphasized, however, that this is a retrospective study and one cannot know whether there were selection factors leading to treatment with ritodrine, which might also be related to risk of RDS. Similarly the results of Kero *et al.* (1973) and Liu (1973), using drugs with related mechanisms of action give only impressions rather than firm conclusions. Prospective, adequately controlled studies are needed to substantiate our results.

There is no difficulty in proposing possible mechanisms of action for a beta-mimetic agent in the prophylaxis of RDS. Fetal hypoxia has been reported to be a predisposing factor to RDS (James, 1959; Miller, 1962), and ritodrine has been reported in a well-controlled study to be effective in the treatment of acute fetal acidosis during labour (Gamissans *et al.*, 1971).

It has been suggested that, as a consequence of the hypoxia, pulmonary vasoconstriction occurs which results in damage to the alveolar epithelial cells responsible for surfactant synthesis (Keuth, 1971). The effect of another beta adrenergic stimulant—isoprenaline—of increasing pulmonary blood flow in the sheep fetus raises a theoretical mechanism of action for this beta-mimetic agent, ritodrine, in the human (Dawes, 1968).

Holm (1973) has made the interesting suggestion that the type of catecholamine response to stress is different in the premature and mature infant, being predominantly noradrenaline mediated in the premature infant (alpha responses) but with more beta-receptor involvement in the mature infant. This difference is presumed to relate to the required cardiovascular responses best suited for continued survival under adverse circumstances in the two very different (*in utero* and extra uterine) environments. A premature infant may thus be born with inappropriate stress responses for independent existence. Beta-mimetic agents could conceivably counteract the excessive alpha responses in the premature neonate.

Gluck and Kulovich (1973) proposed that stress itself may stimulate lung maturation. The relationship between the response to a beta-mimetic agent and the "fight or flight" response to stress is apparent and one might also envisage that, by administration of ritodrine, we simply produced in our series "simulated stress" and evoked the responses proposed by Gluck and Kulovich (1973).

Although ritodrine has been investigated in two well-controlled clinical trials neither of them reports the incidence of RDS in unsuccessfully treated cases (Wesselius-de Casparis *et al.*, 1971; Sivasamboo, 1973). We await with great interest the results of large scale controlled studies in premature labour which might confirm or refute our findings.

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# SECONDARY POSTPARTUM HAEMORRHAGE

BY

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## Summary

The clinical features of 106 women who had a secondary postpartum haemorrhage were examined in detail; 39 were managed conservatively, 3 had a vaginal laceration resutured and 64 had a curettage. In 46 patients, material obtained by curettage was examined histologically. The clinical features associated with retained products of conception are defined and comparisons are made between conservative and surgical management of secondary postpartum haemorrhage.

SECONDARY *postpartum* haemorrhage is any abnormal or excessive bleeding from the birth canal which occurs between 10 hours (Moir and Myerscough, 1971) and 12 weeks (Melody, 1949) after delivery.

This paper reviews and correlates the clinical, bacteriological and histological features of 106 patients who suffered from secondary postpartum haemorrhage, with a view to identifying those who are most likely to have retained products of conception. The results of treatment by curettage and by conservative methods are also employed.

## PATIENTS AND METHODS

In 1971 a total of 8230 patients was delivered at the Royal Women's Hospital, Melbourne. One hundred and six of these patients had excessive or abnormal bleeding between 10 hours and 12 weeks after delivery. Cervical or vaginal smears and cultures were obtained in 82 of the patients. The smears were examined after treatment by Gram's stain and the swabs were cultured on horse blood agar and in cooked-meat medium. Curettings from 46 patients were examined histologically.

## RESULTS

### *Correlation between histological and clinical or bacteriological findings* (Table I)

The curettings were examined histologically in 46 cases. Retained products of conception were found in 19 (41 per cent) patients. In the remainder the curettings consisted of degenerate or hyalinized decidua and/or endometrium and, in one patient only, of myometrium. In several instances where the operation notes describe removal of "gross placental tissue" only decidua was found at histology. Placental tissue was obtained at curettage for secondary postpartum haemorrhage in two patients thought to have an "empty" uterus after manual removal of the placenta. Membranes were detected in the curettings in two patients who bled heavily at six and eight days *post partum*. Placental polyps were present in another two patients who bled 45 and 70 days after delivery.

Retained products of conception were more commonly associated with the following clinical and bacteriological features (see Table I):

(1) primary postpartum haemorrhage of 600 ml. or more,
(2) suspicion of retained placental tissue after inspection of the placenta,
(3) morbidity in the first 10 days of the puerperium,
(4) shock due to severity of secondary postpartum haemorrhage,
(5) morbidity (a temperature above 38 °C.) or mild pyrexia (a temperature of 37·5 to 38 °C.) at the time of secondary haemorrhage,

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TABLE I

*Analysis of features of 106 patients treated for secondary postpartum haemorrhage (PPH = postpartum haemorrhage; UTI = urinary tract infection)*

| Clinical or bacteriological features | | Number | Per cent | Mode of treatment | | | Histological examination | | | Incidence of retained products in curettings examined (per cent) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Conservative | Episiotomy repair | Curettage | Not done | Retained products present | No retained products found | |
| Blood loss at delivery | 600 ml. or more | 16 | 15·1 | 6 | 0 | 10 | 3 | 5 | 2 | 72 |
| | Under 600 ml. | 90 | 84·9 | 33 | 3 | 54 | 15 | 14 | 25 | 36 |
| Placenta at delivery | ?Incomplete | 7 | 6·6 | 2 | 0 | 5 | 2 | 2 | 1 | 67 |
| | Complete | 99 | 93·4 | 37 | 3 | 59 | 16 | 17 | 26 | 40 |
| Morbidity (temperature above 38 °C.) in the first 10 days of puerperium | Present | 12 | 11·3 | 2 | 0 | 10 | 2 | 4 | 4 | 50 |
| | Not present | 94 | 88·7 | 37 | 3 | 54 | 16 | 15 | 23 | 39 |
| Shock due to secondary PPH | Present | 10 | 9·4 | 3 | 0 | 7 | 1 | 4 | 2 | 67 |
| | Not present | 96 | 91·6 | 36 | 3 | 57 | 17 | 15 | 25 | 38 |
| Fever at the time of secondary PPH | more than 38 °C. | 8 | 7·5 | 2 | 0 | 6 | 0 | 5 | 1 (UTI) | 83 |
| | 37·5–38 °C. | 19 | 17·9 | 11 | 0 | 8 | 1 | 4 | 3 | 57 |
| | None | 79 | 74·6 | 26 | 3 | 50 | 17 | 10 | 23 | 30 |
| Uterine tenderness | Present | 9 | 8·5 | 3 | 0 | 6 | 0 | 5 | 1 | 83 |
| | Not present | 97 | 91·5 | 36 | 3 | 58 | 18 | 14 | 26 | 35 |
| Bacteriological smears and cultures | Positive | 9 | 8·5 | 1 | 0 | 8 | 1 | 4 | 3 | 57 |
| | Negative | 73 | 68·9 | 31 | 2 | 40 | 7 | 15 | 18 | 45 |
| | Not taken | 24 | 22·6 | 7 | 1 | 16 | 10 | 0 | 6 | 0 |
| Interval between delivery and secondary PPH | 0–7 days | 24 | 22·6 | 9 | 2 | 13 | 4 | 2 | 7 | 22 |
| | 8–14 days | 35 | 33·0 | 15 | 1 | 19 | 10 | 4 | 5 | 44 |
| | 15–21 days | 18 | 17·0 | 8 | 0 | 10 | 2 | 4 | 4 | 50 |
| | 22–42 days | 17 | 16·1 | 5 | 0 | 12 | 2 | 5 | 5 | 50 |
| | 43–84 days | 12 | 11·3 | 2 | 0 | 10 | 0 | 4 | 6 | 40 |

A total of 35 patients (33 per cent) required blood transfusion for anaemia.

One patient who had had a stillbirth 12 days previously had a low fibrinogen level (10 mg./100 ml.) at the time of secondary PPH. The plasma fibrinogen had been 133 mg./100 ml. before the onset of labour.

(6) uterine tenderness, and
(7) bacteriological evidence of genital tract infection.

The strongest association was between retained products of conception and morbidity at the time of the secondary haemorrhage.

Twenty-four patients had one or more of the clinical or bacteriological features enumerated above and had curettings examined histologically. Retained products of conception were present in 14 (58 per cent) of this group, but in only 5 (23 per cent) of 22 patients without any of these features. The difference was statistically significant ($p<0\cdot05$). Patients with retained products of conception were transfused with an average of 2·7 units of blood, while those showing no histological evidence of retained products received an average of 1·0 unit.

### *Treatment*

Thirty-nine patients (37 per cent) were managed conservatively with bed rest, 0·2 mg. tablets of ergometrine maleate three times daily and antibiotics (usually penicillin and sulphonamides). The other 67 patients (63 per cent) were treated surgically. Three of these simply required resuture of an episiotomy and the remaining 64 had a curettage.

The average bed stay for patients who underwent curettage was 3·8 days as against 3·1 days for patients managed conservatively. Recurrent haemorrhage occurred in only two patients who were managed conservatively. Both eventually had a curettage but placental tissue was only recovered from one of them.

Curettage was accompanied by haemorrhage in five instances, necessitating bimanual compression of the uterus and oxytocics and, on one occasion, packing of the uterus. Perforation of the uterus occurred in one patient who was febrile at the time of operation, 23 days after delivery, when placental tissue was removed from the uterus. Perforation of the uterus was not suspected at the time, but signs of pelvic peritonitis developed postoperatively, to resolve slowly with conservative treatment. The perforation was confirmed two years later when hysterectomy was performed for menorrhagia and pain. Four patients suffered further haemorrhage after curettage; three patients settled with conservative treatment, but the fourth had another curettage with recovery of more placental tissue.

Chronic pelvic infection was diagnosed on clinical and bacteriological grounds in three patients between three and six months after their secondary haemorrhage; all three had had a curettage.

## COMMENTS

The incidence of secondary postpartum haemorrhage in this series was 1·29 per cent. Products of conception were found in the uterus up to 12 weeks after delivery in 19 of the 46 (41 per cent) patients in whom curettings were examined histologically.

Conservative treatment, as applied to 39 patients in this series, was very effective and was attended by remarkably few complications. No doubt retained products of conception were present in a few of them but this appeared to be of little consequence, and such tissue was probably either autolyzed or lost in the lochia or menses.

Curettage was also effective but was associated with more complications. Ultrasonography can help to select patients for curettage. In the series of Malvern *et al.* (1973) 40 patients were curetted after ultrasonography. Retained products of conception were predicted in 25 patients, but in only 8 was the prediction correct because decidua and organizing blood clot had the same ultrasonic appearance as placental tissue. Ultrasonography was more accurate in excluding retained products of conception giving a false negative picture in only two of 15 patients.

In spite of the satisfactory progress shown by patients treated conservatively, those with one or more of the clinical features commonly seen with retained products of conception should have a curettage. The remainder can be effectively and safely managed conservatively unless the bleeding persists or recurs.

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# PREGNANCY IN PATIENTS WITH COELIAC DISEASE

BY

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## Summary

Successful pregnancy can occur both before and after the diagnosis and treatment of gluten-sensitive enteropathy. Abortion and intrauterine growth retardation are the main associated obstetric problems but their incidence may be reduced in the more severe cases by adherence to a strict gluten-free diet and dietary supplements. The present report concerns 60 pregnancies in 25 patients with coeliac disease.

It has been reported that infertility and spontaneous abortion in patients with coeliac disease may be prevented by good dietary management (Morris *et al.*, 1970; Joske and Martin, 1971), but there is remarkably little literature concerning the course and management of pregnancy. Some patients who are diagnosed and treated in childhood return to a normal diet, until there is an unexpected recurrence of symptoms, or an unsuccessful pregnancy, These circumstances provide the opportunity to compare the course and outcome of pregnancy with and without treatment using controlled diet and supportive obstetric care.

Twenty-five patients have been studied at Hammersmith Hospital. They had 38 pregnancies before and 22 pregnancies after commencement of gluten-free diets. In every case the diagnosis had been confirmed by a jejunal biopsy showing total or subtotal villous atrophy.

## MANAGEMENT

### *General management*

Most of the pregnancies reviewed took place in the period 1965 to 1973 during which time the following principles of management were evolved.

Serial measurements were made of maternal weight at each visit, of haemoglobin, serum iron and calcium each month, and of vitamin $B_{12}$ and folate levels to detect any deficiencies.

Adherence to a strict gluten-free diet was thought to be of great importance. Carbohydrate supplements were encouraged, aiming at an extra 400 calories daily. "Hycal", containing dextrans with molecular weights up to 50 000, had been found suitable when glucose tablets were poorly tolerated.

Calcium gluconate may be given twice daily orally in a dose of 1·5 g., adjusted to maintain a normal plasma calcium. Additional iron as ferrous sulphate, 200 mg. twice daily, and folic acid, 5 mg. twice daily, were recommended. If these supplements of iron and folic acid did not produce normal serum levels intramuscular preparations were used and this was also necessary in some patients with coeliac disease who claimed that medication in tablet form increased diarrhoea.

Serum vitamin $B_{12}$ levels were commonly low and cyanocobalamin was given intramuscularly three times during pregnancy, in a dose of 1000 $\mu$g. at 6, 20 and 34 weeks gestation.

In all but the mildest cases in remission, compound intramuscular vitamins were given weekly; a typical regime provided 100 mg. of thiamine hydrochloride, 4 mg. of riboflavine, 50 mg. of pyridoxine hydrochloride, 160 mg. of nicotinamide, 50 mg. of pantothenic acid and 500 mg. of ascorbic acid.

Exacerbations of diarrhoea were controlled by hospital rest, dietary supervision and salicylazosulphapyridine (Salazopyrin) or codeine phosphate as necessary.

### *Obstetric management*

Vaginal cytology and plasma progesterone estimations were used to assess progesterone status in the first 16 weeks of pregnancy because of the increased risk of spontaneous abortion. If the vaginal smear showed a poor progesterone effect 250 mg. of hydroxyprogesterone caproate was given twice a week until the 16th week of pregnancy.

Intrauterine fetal growth retardation was common in this series of patients with coeliac disease. Serial estimations of urinary or plasma oestrogens, plasma progesterone and human placental lactogen together with observations of maternal weight gain (Elder *et al.*, 1970) were of value in the third trimester for monitoring fetoplacental function and deciding the optimum time for delivery of a small-for-dates baby. Similarly, close monitoring of the fetus was maintained in labour.

## RESULTS

### *Biochemical findings*

Representative observations made on five of the patients are shown in Table I. The tendency to deficient absorption of iron, folate and vitamin $B_{12}$ and consequent anaemia will be noted. Occasional low calcium levels occurred but in general the supplements provided were adequate; alkaline phosphatase values were sporadically elevated.

### *Patients on unrestricted diet*

In 38 pregnancies occurring before the diagnosis or before the treatment of coeliac disease there were eight first trimester abortions (21 per cent) but no mid-trimester abortions.

Six babies (16 per cent) were small-for-dates with birthweights below the 10th percentile as defined by Elder *et al.* (1970). One of these, weighing only 1260 g., died from antepartum asphyxia at 33 weeks maturity. Another, weighing 1370 g., was delivered by Caesarean section at 35 weeks because of severe dysmaturity, but died on the fifth day from hyaline membrane disease.

There was one premature labour at 30 weeks gestation in a patient with a very low serum folate (1 ng./ml.) and low serum iron (48 μg./100ml.). The baby weighed 1590 g., survived, and made normal progress. There were 23 other babies whose birthweight was normal for the gestational age. Three patients whose coeliac disease was asymptomatic and not being treated had a total of 9 babies of normal weight. One baby of normal birthweight died an unexplained "cot death" at the age of 10 weeks.

The perinatal mortality in patients on an unrestricted diet was 7 per cent. Twenty-eight out of 38 pregnancies (74 per cent) ended in the birth of a viable infant which survived the neonatal period (Table II).

### *Patients under treatment*

The treatment regime described above was developed partly as a result of experience in managing these patients, and was not implemented in full in all cases.

There was only one spontaneous first trimester abortion and no cases of premature labour in 22 pregnancies in patients adhering to a strict gluten-free diet.

There were 4 small-for-dates babies in the 21 treated patients (19 per cent) delivered after 28 weeks. One was an anencephalic stillbirth weighing 860 g. at 30 weeks gestation, and another was an undiagnosed hydrocephalic delivered by Caesarean section at term because of fetal distress. This baby eventually died at the age of six months.

Of the 17 babies of normal weight, 16 survived; one died of antepartum anoxia at 39 weeks maturity, the loss being ascribed to placental insufficiency.

The perinatal mortality rate in the treated group was 10 per cent. Nineteen out of 22 pregnancies (86 per cent) ended in the birth of a viable infant which survived the neonatal period (Table II).

### *Maternal weight changes*

At the start of pregnancy the patient's weights were within the range 47·6 to 59·4 kg. They all gained weight during pregnancy, the average total gain being 9·1 kg.

In 16 patients who delivered normal weight babies, the mean maternal weight gain was 12·3 kg. Between 34 weeks maturity and the time of delivery, 12 of the 16 patients gained

TABLE I

*Representative haematological and biochemical observations in five patients with coeliac disease*

| | Normal range | Unrestricted diet | | | | Gluten-free diet | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Between pregnancies | | During pregnancy | | Between pregnancies | | During pregnancy | |
| | | Patient 1 | Patient 2 | Patient 1 | Patient 3 | Patient 4 | Patient 3 | Patient 2 | Patient 5 |
| Haemoglobin (g.) | | 13·1 | 12·0 | 8·6* | 10·8* | 11·1 | 11·3 | 11·5 | 12·6 |
| White blood cells (per cu.mm.) | | 10 000 | – | 18 500* | 17 000* | 6 100 | 3 000 | 10 400 | – |
| E.S.R. (Wintrobe: mm./hour) | | 4 | 5 | 54* | – | 55* | 10 | – | 4 |
| Serum vitamin B12 (pg./ml.) | 160–900 | 270 | 280 | 160 | 136* | 60* | 265 | 368 | 384 |
| Serum folate (ng./ml.) | 3–21 | 1·8* | 2·0* | 1·4* | 1·8* | 0·8* | 3·2 | 3·6 | 6·4 |
| Red cell folate (ng./ml.) | 160–640 | – | – | 75* | – | 62* | – | 253 | 415 |
| Serum iron (μg./100 ml.) | 110–130 | 110 | 20* | 20* | 26* | 48* | 80* | 50* | 68* |
| Serum calcium (mg./100 ml.) | 8·5–10·5 | 9·0 | 8·1* | 8·5 | 9·0 | 8·6 | 9·4 | 8·6 | 9·5 |
| Serum inorganic phosphorus (mg.) | 2·5–4·5 | 3·5 | 3·9 | 2·5 | 3·7 | 2·0* | 4·5 | 2·6 | 4·0 |
| Serum alkaline phosphatase (IU/1) | 30–130 | 70 | 26 | 100 | 187* | 288* | 90 | 132* | 60 |
| Serum albumin (g.) | | 4·4 | 4·2 | 4·0 | 3·7 | 2·2* | 4·0 | 4·6 | 4·5 |
| Serum globulin (g.) | | 3·6 | 2·9 | 3·0 | 3·3 | 3·2 | 2·3* | 2·4 | 3·0 |
| Blood urea (mg.) | | 21 | – | 16 | 21 | 23 | 20 | 14 | 22 |

*Abnormal values. When several values were recorded the most abnormal is given

1 to 3·3 kg., the mean gain after 34 weeks for the 16 patients being 2·3 kg.

In 10 patients who delivered small-for-dates babies, the mean maternal weight gain in pregnancy was 5·9 kg. and only 4 of these gained any weight after 34 weeks maturity.

### *Outcome of pregnancy (Table II)*

No significant differences were demonstrable at the P = 0·05 level between the treated and untreated groups, but the apparent overall improvement in outcome with treatment is encouraging.

None of the babies was found to have coeliac disease. Plasma magnesium and calcium levels in the cord blood were at the lower limit of normal in two babies, but both made normal progress and required no treatment.

TABLE II

*Details of 60 pregnancies in 25 patients with coeliac disease*

| | Before treatment | After treatment | Totals |
|---|---|---|---|
| Total pregnancies | 38 | 22 | 60 |
| Abortions | 8 (21%) | 1 (4%) | 9 (15%) |
| Baby small-for-dates | 6 (16%) | 4*† (18%) | 10 (17%) |
| Normal birthweight | 24 (63%) | 17 (78%) | 41 (68%) |
| Stillbirths | 1 | 2* | 3 |
| Neonatal deaths | 1 | 0 | 1 |
| Infant deaths | 1 | 1† | 2 |
| Perinatal mortality | 7% | 10%* | 8% |
| Pregnancies ending in the birth of a viable infant which survived 28 days | 74% | 86% | 78% |

*1 anencephalic fetus
†1 hydrocephalic fetus

## DISCUSSION

Infertility is an occasional feature of active coeliac disease. All three such patients described by Morris *et al.* (1970) became pregnant when their symptoms were controlled by dietary gluten restriction. None of the 25 patients in this series had complained of infertility.

Spontaneous abortion has been shown to be increased in patients with low serum folate levels (Martin *et al.*, 1965) which may explain the 20 per cent spontaneous abortion rate in our untreated patients. Unrecognized coeliac disease is a rare cause of recurrent first trimester abortion. Joske and Martin (1971) studied 21 patients with at least three consecutive first trimester miscarriages. Three had low serum folate levels and two of these were found to have previously unrecognized coeliac disease. Treatment with a gluten-free diet was followed by normal pregnancies in both of these patients.

It has been suggested that conception at a time of low folic acid status might be associated with an increased incidence of fetal abnormalities (Hibbard and Smithells, 1965). In the present study, these were two abnormal infants and both were born to treated patients in whom folic acid deficiency was less likely than in the untreated patients.

A strict gluten-free diet, laboratory assessment and correction of deficiencies and serial assessment of placental function seem to improve the outcome of pregnancy in women with coeliac disease.

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# SERUM LIPIDS IN PREGNANCY AND SOCIO-ECONOMIC STATUS

BY

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## Summary

Serum total and free cholesterol, phospholipids and erythrocyte free cholesterol levels were determined at four-weekly intervals from the first trimester until term in pregnant Nigerian women from three different socio-economic levels. The results showed a similar pattern of serum lipids in all three groups with a similar percentage increase at the peak levels. Serum phospholipids and total cholesterol levels were not significantly increased after the second trimester in contrast to reports of a progressive hyperlipaemia in pregnant women of Western countries. Erythrocyte free cholesterol levels showed a mirror image of those of serum free cholesterol.

IN a previous study of serum lipids and fatty acid composition in pregnant Nigerian women, it was shown that the hyperlipaemia associated with pregnancy was less pronounced in Nigerian women compared with women from Western countries (Taylor, 1972). The suggestion was made that this difference may be due to an inadequate calorie intake since the Nigerian women who were studied were from the low income class and their level of nutrition was known to be inadequate. The present study was therefore undertaken to determine whether socio-economic status has any effect on the levels of serum lipids in pregnancy. The study was extended to include changes in serum lipids as early as ten weeks of pregnancy and at four weekly intervals up till term and also to determine whether a relationship existed between the levels of serum and erythrocyte free cholesterol.

## METHODS

The subjects studied were all booked patients attending the antenatal clinic at the University College Hospital, Ibadan. All those who had hypertension, diabetes mellitus, chronic nephritis and pre-eclampsia were excluded from the study. Those who did not know the exact date of their last menstrual periods were also excluded. The study was commenced in the first trimester and continued throughout pregnancy in a few subjects whilst in others blood samples were collected at various stages of gestation for only part of the pregnancy. Five or more consecutive samples were obtained in about a quarter of the latter group of subjects.

The subjects were divided into three income groups—low, medium and high—depending on the combined annual income of the patients and their husbands. Those whose combined annual income was below ₦1000 (£600) were classified as of low income status (Group III), those between ₦1000 and ₦2000 (£1200) were classified as middle income status (Group II) whilst those above ₦2000 (£1200) were classified as high income status (Group I).

Venous blood was collected after an overnight fast into a sequestrene tube and also a plain universal bottle. Serum was extracted from the clotted blood for determination of total and free cholesterol by the method of Ferro and Ham (1960), and phospholipids by the method of King and Wootton (1956). Free cholesterol in whole blood was determined by the same method as that used for serum free cholesterol in serum while erythrocyte free cholesterol values were calculated from the values of the serum free cholesterol and those of packed cell volume (PCV).

## RESULTS

The mean values for serum total and free cholesterol, phospholipids and erythrocyte free cholesterol at all stages of pregnancy and at the maximum or minimum values of these variables are shown in Tables I to IV. The mean serum total cholesterol level at the beginning of pregnancy was significantly different between Groups I and III (P<0·05) but not between Groups I and II. Similarly at the maximum cholesterol level, the mean for Group I was not significantly different from that of Group II but was significantly higher than the mean for Group III (P<0·001). The maximum increase in the total cholesterol throughout pregnancy for Groups I, II and III were 24·3, 25·0 and 26·0 per cent respectively.

The mean serum free cholesterol level showed a maximum increase of 39, 25·9 and 35·8 per cent for Groups I, II and III respectively (Table II; Fig. 2).

The maximum increases in the phospholipid levels were 23·1, 13·7 and 23·0 per cent for Groups I, II and III respectively (Table III). The pattern of increase of the serum phospholipids in Groups I and II were similar (Fig. 3). Figure 4 shows that there was a progressive decrease in erythrocyte free cholesterol in the three groups.

TABLE I

*Serum total cholesterol at varying gestational ages in groups I, II and III*

| | Gestational age (weeks) | No. of patients | Serum total cholesterol (mg./100 ml.) | | | Comparison with level at 0–12 weeks* |
|---|---|---|---|---|---|---|
| | | | Mean | S.D. | S.E. | |
| Group I | 0–12 | 11 | 208·3 | 53·1 | 16·1 | |
| | 13–16 | 13 | 226·1 | 41·3 | 11·5 | |
| | 17–20 | 10 | 226·9 | 32·4 | 10·3 | |
| | 21–24 | 7 | 231·5 | 15·0 | 6·7 | |
| | 25–28 | 4 | 210·5 | 12·7 | 6·3 | |
| | 29–32 | 5 | 259·0 | 25·1 | 11·3 | 24·3 per cent increase |
| | 33–36 | 3 | 227·3 | 5·9 | 3·4 | |
| | 37–40 | – | – | – | – | |
| Group II | 0–12 | 19 | 183 0 | 34·7 | 8·9 | |
| | 13–16 | 21 | 197·2 | 40·2 | 8·8 | |
| | 17–20 | 31 | 212·3 | 33·9 | 6·1 | |
| | 21–24 | 35 | 211·4 | 24·9 | 4·2 | |
| | 25–28 | 16 | 230·0 | 28·1 | 6·5 | |
| | 29–32 | 15 | 214·3 | 36 5 | 9 4 | 25·5 per cent increase |
| | 33–36 | 19 | 203 1 | 35 0 | 8·1 | |
| | 37–40 | 2 | 176·0 | 11·1 | 8·0 | |
| Group III | 0–12 | 12 | 165·6 | 39·6 | 11·4 | |
| | 13–16 | 24 | 174·8 | 25·9 | 5·3 | |
| | 17–20 | 16 | 207·0 | 20 8 | 5·2 | |
| | 21–24 | 21 | 195·8 | 28·4 | 6·2 | |
| | 25–28 | 10 | 208·7 | 20·6 | 5·0 | |
| | 29–32 | 5 | 209·0 | 15·5 | 7·0 | 26·0 per cent increase |
| | 33–36 | 9 | 205 4 | 26·6 | 8·9 | |
| | 37–40 | 2 | 209·8 | 16·2 | 11 5 | |

*Only maximum change is indicated.

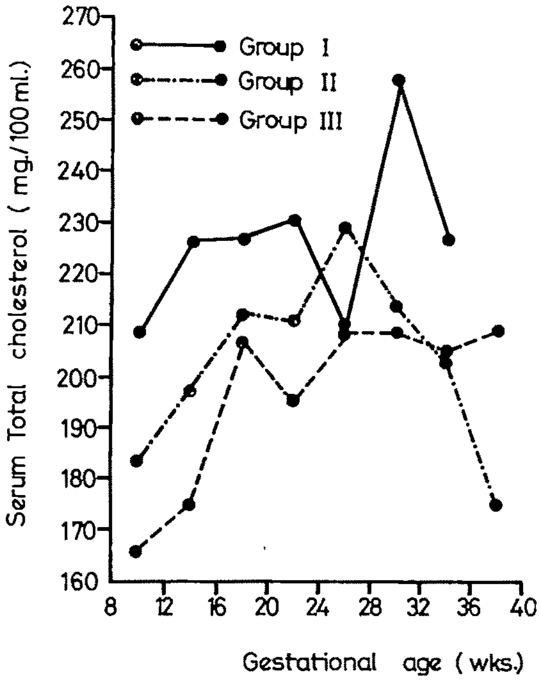

Fig. 1

Mean serum total cholesterol levels at varying gestational ages in pregnant Nigerian women from three different socio-economic levels.

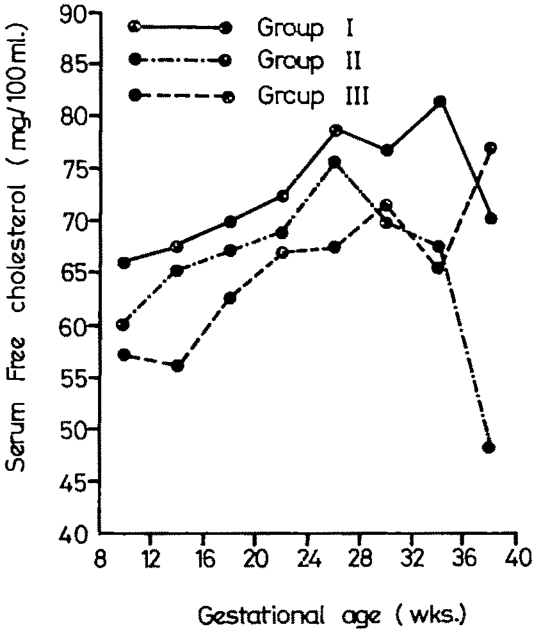

Fig. 2

Mean serum free cholesterol levels at varying gestational ages in pregnant Nigerian women from three different socio-economic levels.

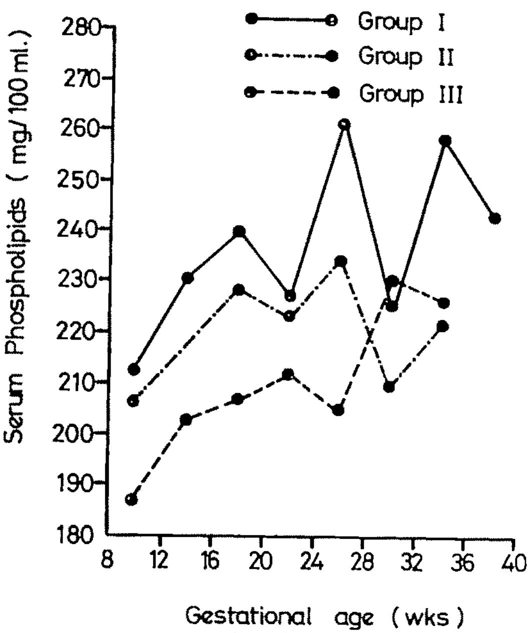

Fig. 3

Mean serum phospholipid levels at varying gestational ages in pregnant Nigerian women from three different socio-economic levels.

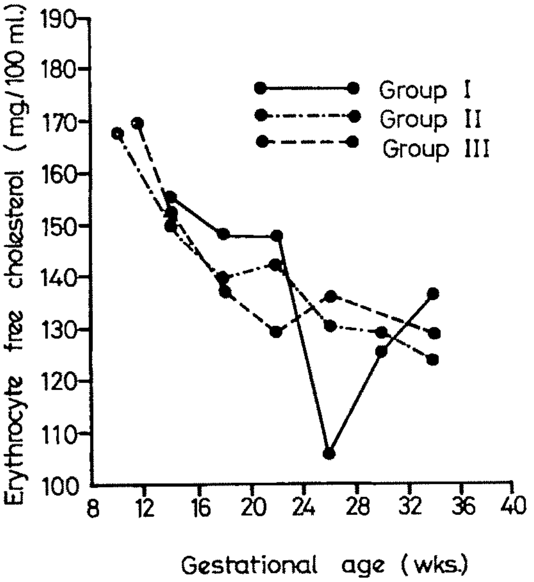

Fig. 4

Mean erythrocyte free cholesterol levels at varying gestational ages in pregnant Nigerian women from three different socio-economic levels.

TABLE II

*Serum free cholesterol at various gestational ages in groups I, II and III*

| | Gestational age (weeks) | No. of patients | Serum free cholesterol (mg./100 ml.) | | | Comparison with level at 0–12 weeks* |
|---|---|---|---|---|---|---|
| | | | Mean | S.D. | S.E. | |
| Group I | 0–12 | 11 | 66·2 | 7·4 | 3·3 | 39 0 per cent increase |
| | 13–16 | 11 | 68·6 | 10·6 | 3·2 | |
| | 17–20 | 12 | 70·0 | 10·0 | 2·9 | |
| | 21–24 | 11 | 72·6 | 10·1 | 3·1 | |
| | 25–28 | 6 | 79·0 | 4·3 | 1·7 | |
| | 29–32 | 6 | 77·1 | 13·6 | 5·1 | |
| | 33–36 | 5 | 82·0 | 12·6 | 5·6 | |
| | 37–40 | 3 | 70·6 | 3·3 | 1·9 | |
| Group II | 0–12 | 19 | 60·2 | 10·5 | 2·4 | 25·9 per cent increase |
| | 13–16 | 22 | 65·3 | 9·9 | 2·1 | |
| | 17–20 | 29 | 67·5 | 9·9 | 1·8 | |
| | 21–24 | 35 | 69·1 | 9·4 | 1·6 | |
| | 25–28 | 18 | 75 8 | 11·2 | 2·1 | |
| | 29–32 | 16 | 70·2 | 15·3 | 3·8 | |
| | 33–36 | 19 | 68·0 | 12·8 | 3·0 | |
| | 37–40 | 2 | 48·3 | 2·4 | 1·7 | |
| Group III | 0–12 | 12 | 57·1 | 13·0 | 3·6 | 35·4 per cent increase |
| | 13–16 | 25 | 56 0 | 12·0 | 2·3 | |
| | 17–20 | 16 | 62·6 | 8·7 | 2·2 | |
| | 21–24 | 22 | 67·1 | 11·6 | 2·5 | |
| | 25–28 | 17 | 67·7 | 11·0 | 2·7 | |
| | 29–32 | 10 | 72·0 | 8·7 | 2·9 | |
| | 33–36 | 9 | 66·0 | 10·5 | 3·5 | |
| | 37–40 | – | – | – | – | |

*Only maximum change is indicated.

TABLE III

*Serum phospholipids at varying gestational ages in groups I, II and III*

| | Gestational age (weeks) | No. of patients | Serum phospholipids (mg./100 ml.) | | | Comparison with level at 0–12 weeks* |
|---|---|---|---|---|---|---|
| | | | Mean | S.D. | S.E. | |
| Group I | 0–12 | 11 | 213·0 | 44·9 | 22·5 | 23 1 per cent increase |
| | 13–16 | 11 | 231·0 | 40·4 | 12·2 | |
| | 17–20 | 12 | 240·3 | 36·2 | 10 5 | |
| | 21–24 | 9 | 227·6 | 29·1 | 9·7 | |
| | 25–28 | 5 | 261·0 | 17·0 | 6·4 | |
| | 29–32 | 5 | 226·0 | 44·3 | 10·8 | |
| | 33–36 | 4 | 259·5 | 11·5 | 5·7 | |
| | 37–40 | 2 | 244·4 | 6 4 | 4 5 | |
| Group II | 0–12 | 19 | 206·0 | 41·3 | 9·2 | 13·7 per cent increase |
| | 13–16 | 18 | 216·4 | 30·6 | 7·2 | |
| | 17–20 | 29 | 229·8 | 39·9 | 7·4 | |
| | 21–24 | 31 | 223·8 | 40·2 | 7·2 | |
| | 25–28 | 16 | 234·7 | 20 5 | 5·9 | |
| | 29–32 | 14 | 209·7 | 26 8 | 7·2 | |
| | 33–36 | 15 | 222·0 | 28·8 | 7·4 | |
| | 37–40 | — | – | – | – | |
| Group III | 0–12 | 12 | 187 2 | 41·2 | 13·0 | 23·0 per cent increase |
| | 13–16 | 24 | 203·3 | 47·9 | 9·8 | |
| | 17–20 | 12 | 207·1 | 38·3 | 11·1 | |
| | 21–24 | 22 | 212 4 | 33·3 | 7·1 | |
| | 25–28 | 15 | 205·8 | 35·9 | 10·3 | |
| | 29–32 | 10 | 231·0 | 42·3 | 21·2 | |
| | 33–36 | 9 | 227·0 | 33·2 | 11 1 | |
| | 37–40 | – | – | – | – | |

*Only maximum change is indicated.

TABLE IV
*Erythrocyte free cholesterol at varying gestational ages in groups I, II and III*

| | Gestational age (weeks) | No. of patients | Erythrocyte free cholesterol (mg./100 ml.) | | | Comparison with level at 0–12 weeks* |
|---|---|---|---|---|---|---|
| | | | Mean | S.D. | S.E. | |
| Group I | 0–12 | – | – | – | – | |
| | 13–16 | 11 | 156·0 | 14·0 | 7·0 | |
| | 17–20 | 8 | 149·0 | 23·0 | 8·0 | |
| | 21–24 | 4 | 149·0 | 20·0 | 10·0 | |
| | 25–28 | 5 | 106·0 | 11·0 | 6·3 | |
| | 29–32 | 3 | 126·0 | 12·0 | 7·1 | 25·6 per cent decrease |
| | 33–36 | 2 | 137·2 | 5·0 | 3·3 | |
| | 37–40 | – | – | – | – | |
| Group II | 0–12 | 8 | 167·8 | 23·5 | 8·9 | |
| | 13–16 | 9 | 150·1 | 20·4 | 7·8 | |
| | 17–20 | 20 | 140·4 | 27·3 | 6·1 | |
| | 21–24 | 26 | 143·1 | 26·8 | 5·0 | |
| | 25–28 | 14 | 131·3 | 24·6 | 6·3 | |
| | 29–32 | 9 | 129·7 | 32·1 | 11·7 | 23·4 per cent decrease |
| | 33–36 | 9 | 137·1 | 25·8 | 8·2 | |
| | 37–40 | – | – | – | – | |
| Group III | 0–12 | 6 | 170·4 | 10·8 | 5·9 | |
| | 13–16 | 12 | 153·2 | 15·8 | 4·2 | |
| | 17–20 | 11 | 137·2 | 22·5 | 6·4 | |
| | 21–24 | 13 | 130·5 | 22·5 | 6·9 | |
| | 25–28 | 10 | 137·6 | 24·3 | 8·6 | |
| | 29–32 | – | – | – | – | |
| | 33–36 | 10 | 124·4 | 8·8 | 3·9 | 27·0 per cent decrease |
| | 37–40 | – | – | – | – | |

*Only maximum change is indicated.

## DISCUSSION

The present study has shown that a definite pattern of change occurred in serum lipid levels in pregnancy. These changes were not observed in a previous study (Taylor, 1972) where determination of serum lipids was only performed at the 24th and 36th weeks of pregnancy. The present work has thus filled the gaps left in that study. The values for total and free cholesterol and phospholipids obtained for the Group III subjects in the present study are similar to those previously reported for the low socio-economic group (Taylor, 1972). There was very little increase in serum phospholipids and total cholesterol after the second trimester of pregnancy.

The role of socio-economic factors in the changes in serum lipid levels during pregnancy has been the subject of considerable studies. Mullick (1964) in a comprehensive study of serum lipids of pregnant women in New Delhi found significantly higher total lipids, total cholesterol and $\alpha/\beta$ lipoprotein ratios in the sera of the women of high socio-economic status and noted that the serum lipid values of vegetarian and non-vegetarian women of the low income group were almost identical during the third trimester. He concluded that diet had no significant influence on lipid synthesis in the later period of pregnancy. Similar conclusions were drawn by Hansen *et al.* (1964) who found no significant correlation between the mother's intake of calories, protein, fat and fatty acids and their serum cholesterol and fatty acid levels during the third trimester.

Hormonal factors have been implicated in the changes which occur in serum lipids in pregnancy (Venning, 1955; Jailer and Longson, 1957). De Alvarez *et al.* (1967) showed that similar changes in serum lipids occur in pregnancy as well as in gynaecological cancer. These authors attributed the changes to common factors of stress and growth which could be trophoblastic or neoplastic in origin. They put forward the hypothesis that the alpha cells of the anterior pituitary gland responding to stress induced by pregnancy or malignancy react by secreting growth hormone to enhance protein metabolism and, by its diabetogenic effect, decrease carbohydrate utilization, resulting in mobilization of fat to provide energy. Adrenocorticotrophic hormone (ACTH) which is simultaneously activated promotes the release of glucocorticoids, which in turn stimulates the pituitary output of lipid mobilizer hormone which acting on depot fat (subcutaneous depots, omental mesenteric fat) releases into the circulation non-esterified fatty acids and triglycerides with simultaneously increased ketogenesis. Studies on plasma cortisol level in the pregnant Nigerian women have shown no significant increase in cortisol levels in the second and third trimesters (Adadevoh and Akinla, 1971). This may have a bearing on our findings which also show that serum phospholipids and cholesterol levels are not significantly increased after the second trimester.

The present study has not revealed any differences in the percentage increase of serum lipid levels in the three socio-economic groups. This finding has not therefore substantiated the suggestion that dietary factors could account for the smaller increase in serum lipids in pregnant Nigerian women of low socio-economic status as compared with women of Western countries (Taylor, 1972). The absence of a progressive hyperlipaemia in pregnant Nigerian women may be partly explained by the poor response of the adrenal cortex to the stress of pregnancy as shown by Adadevoh and Akinla (1971).

It is also worthy of note that erythrocyte free cholesterol levels showed a mirror image of the values obtained in the serum. The changes in serum levels would therefore appear to be due to a shift from the cell to the serum.

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# THE PROGNOSTIC VALUE OF HUMAN PLACENTAL LACTOGEN (HPL) LEVELS IN THREATENED ABORTION

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## Summary

The value of a single HPL estimation in serum was assessed in 337 patients with a threatened abortion between 7 and 27 weeks gestation. Serial assays were subsequently performed on 75 of these. A scheme is proposed whereby an HPL result can be given a "favourable", "equivocal" or "unfavourable" prognosis according to its level. Using this scheme a correct prognosis was obtained in 86 per cent of cases between 9 and 19 weeks gestation while an incorrect prognosis was obtained in 3 per cent of cases. There appeared to be little prognostic value after 19 weeks gestation though prior to 9 weeks the results were sufficiently promising to suggest that a more sensitive assay would be useful.

FOLLOWING early studies of HPL levels in serum in early pregnancy and abortion (Saxena *et al.*, 1969; Singer *et al.*, 1970; Spona and Janish, 1971; Genazzani *et al.*, 1971 and 1972), major studies have led to its efficacy as a prognostic index being variably reported.

Niven *et al.* (1972) using a semi-automated method of radio-immunoassay claim to predict with "reasonable accuracy" the outcome of pregnancies complicated by vaginal bleeding from the ninth week and suggest serial samples may be of value before then. Ylikorkala and Jouppila (1973) using a Sclava-Sorin HPL radioimmunoassay kit consider that a single HPL estimation is only of value after the 14th week; prior to this serial specimens are required.

The present study confirms the need for an additional test of viability in early pregnancy and establishes a range of normal values using the Radiochemical Centre's HPL kit according to the instructions. Information gained from single and serial specimens in cases of threatened abortion has been evaluated. Since few biological parameters give absolute information some overlap between "favourable" and "unfavourable" results is inevitable. A scheme is proposed whereby the HPL level obtained can give a "favourable", "equivocal" or "unfavourable" prognosis.

## SUBJECTS AND METHODS

The normal range was established on the results of 536 serum samples from 225 uncomplicated pregnancies of certain gestation length. Serial sampling was performed throughout 54 of these pregnancies; all 225 resulted in the delivery of normal infants.

The mean curve for normal pregnancy was devised by taking logarithms of the HPL levels to normalize them and by smoothing the curves. The standard deviations at various periods of gestation were similarly estimated and the lower limit of the "favourable" zone was then set at 1·75 standard deviations below the mean for that period of gestation. The "unfavourable" zone was set at 3 standard deviations below the mean value.

A group of women with threatened abortions was obtained from various hospitals in and around Manchester. In all cases, either bleeding or bleeding and pain had occurred in the first two trimesters of pregnancy. A total of 337 admissions in 325 patients were studied with single serum specimens. Seventy-five of these women subsequently had serial studies performed. Confirmatory evidence of pregnancy was obtained either by positive pregnancy tests, products of conception being passed or evacuated, sonar scan confirmation of pregnancy or clinically a continuing pregnancy. At least one of these criteria was fulfilled in all but 27 cases and these are considered separately. No clinical decision was taken as a result of these assays and they were not known to the clinician.

All assays were performed in duplicate together with a control serum of standard concentration. The coefficient of variation on the latter was 4 per cent.

## RESULTS

### *Outcome of pregnancy*

One hundred and sixty-four pregnancies failed to reach the 28th week; 140 women either aborted spontaneously or had the uterus evacuated of what had subsequently been decided was a non-viable pregnancy during their only admission to hospital. Of the remaining 24, 19 were discharged home but aborted much later in the pregnancy and five were non-viable pregnancies at the time of discharge.

Of the remaining 173 admissions 27 were considered to lack confirmatory evidence of pregnancy, 17 were lost to subsequent follow-up and the remainder resulted in normal pregnancies after the period of viability (28 weeks).

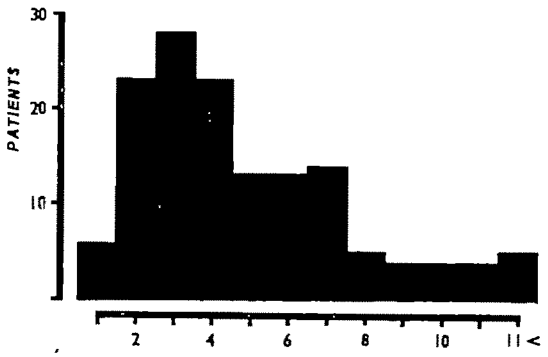

FIG. 1

Days in hospital before abortion or evacuation.

### *Period in hospital*

A considerable time is wasted in hospital prior to abortion or evacuation of a non-viable pregnancy as shown by Figure 1. This shows the number of days or parts of a day spent in hospital prior to either of these events. Even excluding the few atypical cases whose admission exceeded 11 days the average was 4·7 days.

### *Evaluation of HPL estimations*

It is apparent that the prognostic value of an HPL estimation varies according to the period of gestation. The maximum value is between the ninth to nineteenth week. After 19 weeks there was little of prognostic significance in the results and below nine weeks the assay method which was modified as a result of this study was not sufficiently accurate to be meaningful. There was evidence that suggested a more sensitive assay before nine weeks would be of value.

A more detailed assessment of the results is presented in three phases; between 9 and 19 weeks, after 19 weeks and before 9 weeks.

### *9 to 19 weeks gestation*

Table I gives the upper limit of the "un-

TABLE I

*Zones for HPL levels at 9 to 19 weeks gestation*

| Weeks of gestation | Upper limit of unfavourable zone (μg./ml.) | Lower limit favourable zone (μg /ml.) |
|---|---|---|
| 9 | 0·014 | 0·031 |
| 9½ | 0·021 | 0·044 |
| 10 | 0·029 | 0·059 |
| 10½ | 0·040 | 0·078 |
| 11 | 0·053 | 0·10 |
| 11½ | 0·068 | 0·13 |
| 12 | 0·087 | 0·16 |
| 12½ | 0·105 | 0·20 |
| 13 | 0·13 | 0·23 |
| 13½ | 0·15 | 0·28 |
| 14 | 0·18 | 0·33 |
| 14½ | 0·21 | 0·38 |
| 15 | 0·25 | 0·43 |
| 15½ | 0·29 | 0·49 |
| 16 | 0·33 | 0·55 |
| 16½ | 0·38 | 0·61 |
| 17 | 0·43 | 0·68 |
| 17½ | 0·48 | 0·76 |
| 18 | 0·54 | 0·83 |
| 18½ | 0·60 | 0·91 |
| 19 | 0·66 | 0·99 |

favourable" zone and the lower limit of the "favourable" zone for the appropriate period of gestation. Any value between these levels represents an "equivocal" result.

Two hundred and fourteen separate admissions (209 patients) were evaluated on the result of a single assay. In 53 of these repeat assays were usually performed every 3 days until either the patient was allowed home with an intact pregnancy or pregnancy had ended in abortion or evacuation.

Out of the 214 single assays 94 (44 per cent) lay within the "favourable" zone. Out of these 94 pregnancies 90 (96 per cent) were discharged with a potentially normal pregnancy. Sixty-two were known subsequently to reach as far as the 28th week without any further problem, 17 were lost to subsequent follow-up and 11 aborted between 2 and 16 weeks following discharge from hospital. In the remaining four cases (4 per cent) there was an incorrect prognosis, abortion occurring approximately three days after the blood sample had been taken. The gestation lengths of these four cases were 16, 17, 18 and 19 weeks.

HPL levels from 97 patients (45 per cent) admitted to hospital were assigned to the "unfavourable" zone and 86 (88 per cent) of these pregnancies aborted or required evacuation. In five pregnancies in which viability was very much in doubt clinically the patient was allowed home to await events. All five were subsequently readmitted as missed or inevitable abortions.

There were six results in the "unfavourable" zone from viable pregnancies (two were from

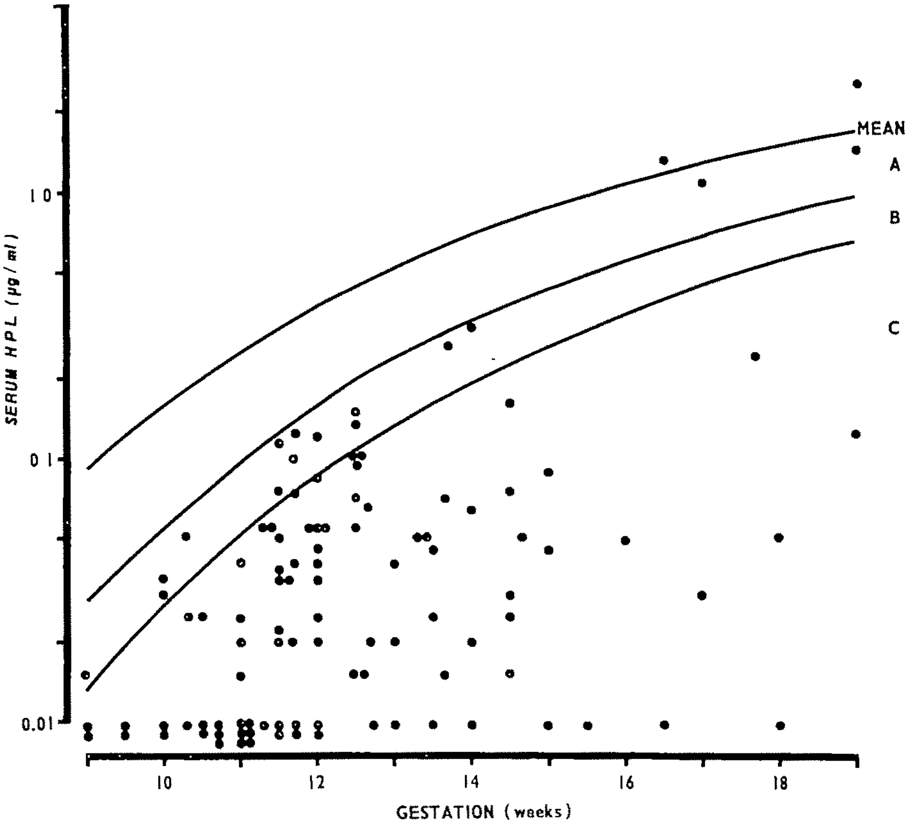

FIG. 2

Individual HPL levels in patients between 9 and 19 weeks gestation with a doomed pregnancy. The zones A, B and C represent the "favourable", "equivocal" and "unfavourable" zones respectively.

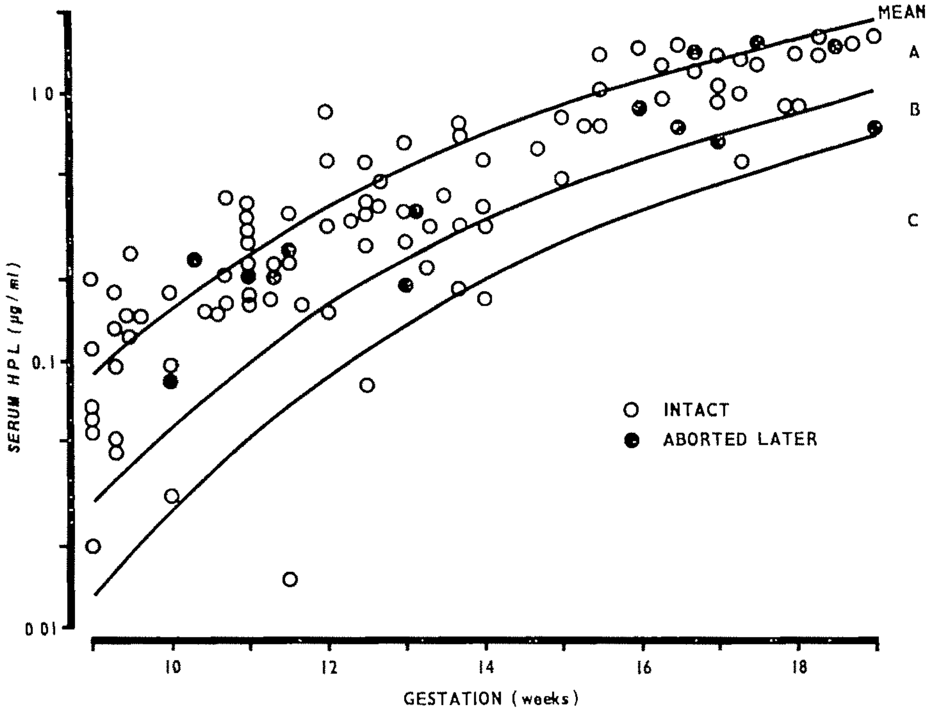

Fig. 3

Individual HPL levels in patients between 9 and 19 weeks gestation discharged with intact pregnancies. The zones A, B and C represent the "favourable" "equivocal" and "unfavourable" zones respectively.

one pregnancy on separate hospital admissions). At subsequent ante-natal visits it became obvious that the period of gestation in three patients was less advanced than originally believed. In these three mothers when the HPL results were correctly matched with the period of gestation a "favourable" prognosis would have been given. The other two pregnancies which gave the remaining three "unfavourable" results (two patients) there was no evidence to suggest uncertain gestation and the result would have given the wrong prognosis if acted on.

Of 23 results in the "equivocal" zone, 13 of the pregnancies were non-viable and 10 women were discharged with intact pregnancies but three subsequently aborted.

Figures 2 and 3 show individual HPL levels in doomed and viable pregnancies respectively.

*A single reading of HPL between 9 and 19 weeks of pregnancy thus appears to give the correct immediate prognosis in 86 per cent of threatened abortion*, an "equivocal" prognosis in 11 per cent of cases and a wrong prognosis in 3 per cent.

The value of serial assays between 9 and 19 weeks gestation was based on 53 hospital admissions (51 patients; see Table I). Out of these, 26 originally had levels which lay within the "favourable" zone and all of this group were discharged with intact pregnancies although four subsequently aborted. Apart from one patient whose result was in the "equivocal" zone followed by a "favourable" result all subsequent assessments remained "favourable".

Sixteen patients whose results were "unfavourable" and later aborted had further "unfavourable" results. Two of the three patients admitted with "unfavourable" values but who in fact had intact pregnancies gave a further "unfavourable" result whilst the other had a result in the "equivocal" range. Of eight patients with "equivocal" readings, four subsequently produced "unfavourable" values and aborted.

TABLE II

*Relationship between prediction made during hospital admission at 9 to 19 weeks gestation and outcome of pregnancy*

| | | No. | Per cent | Outcome of pregnancy about which a prediction was made | | | |
|---|---|---|---|---|---|---|---|
| | | | | Normal | Lost to follow-up | Late abortion | Early abortion |
| Predictions made during 214 admissions with single HPL assay (209 patients) | Favourable | 94 | 44 | 62 | 17 | 11 | 4 |
| | Unfavourable | 97 | 45 | 6 | | 5 | 86 |
| | Equivocal | 23 | 11 | 7 | | 3 | 13 |
| Predictions made during 53 admissions with serial HPL assays (51 patients) | Consistently favourable | 26 | | 22 | | 4 | |
| | Consistently unfavourable | 18 | | 2 | | | 16 |
| | Consistently equivocal | 3 | | 3 | | | |
| | Unfavourable then equivocal | 1 | | 1 | | | |
| | Equivocal then unfavourable | 4 | | | | | 4 |
| | Equivocal then favourable | 1 | | 1 | | | |

Four intact pregnancies resulted and the serial estimates in three remained "equivocal" and the other patient had "favourable" values.

For this group it seems reasonable to conclude that subsequent readings add little to the initial "favourable" or "unfavourable" value but are of use when the result lies in the "equivocal" range. If serial specimens are taken, the majority of patients with intact pregnancies produce a significant increase in HPL levels over a three-day period, whereas the doomed pregnancies tend to produce static or falling levels. This is shown by Figures 4 and 5 and has been previously noted (Genazzani *et al.*, 1971 and 1972; Singer *et al.*, 1970; Ylikorkala *et al.*, 1973).

### *After 19 weeks gestation*

Fifty-eight admissions (53 patients) were studied between 19 and 27 weeks of gestation, 15 of these with serial specimens.

In 53 admissions the results were "favourable" and resulted in 40 intact pregnancies and 13 abortions. Of the 15 pregnancies in which serial specimens were assayed all gave results within

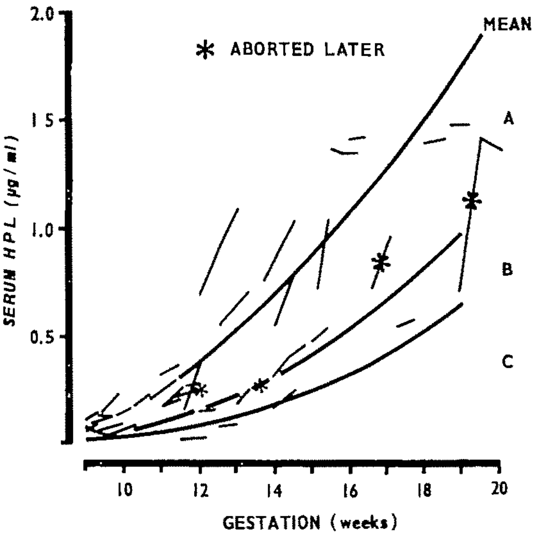

FIG. 4

Serial HPL levels in patients between 9 and 19 weeks gestation with intact pregnancies. The zones A, B and C represent the "favourable", "equivocal" and "unfavourable" zones respectively.

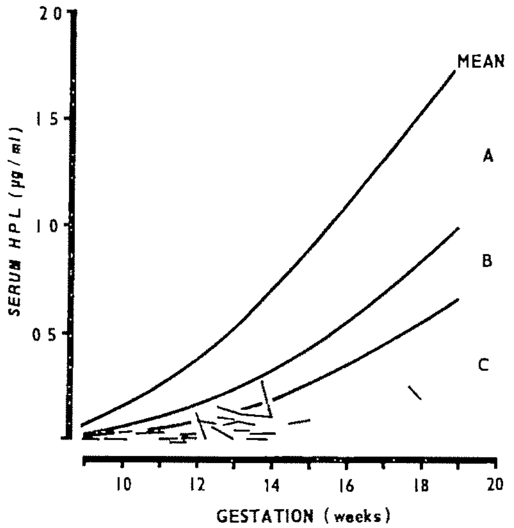

FIG. 5

Serial HPL levels in patients between 9 and 19 weeks gestation with a doomed pregnancy. The zones A, B and C represent the "favourable", "equivocal" and "unfavourable" zones respectively.

the "favourable" zone but five of the pregnancies ended in abortion.

Two pregnancies whose HPL results were "equivocal" resulted in normal pregnancies. Three pregnancies in which the levels were considered to be "unfavourable" all aborted.

There appears to be little of prognostic value in a single "favourable" result or serial "favourable" results after 19 weeks gestation since nearly 25 per cent end in an abortion.

An "unfavourable" result would appear to have more prognostic significance although the evidence for this is scanty. Single and serial levels in post 19 week cases are shown in Figure 6.

*Before 9 weeks gestation*

Thirty-eight admissions were studied, 8 of these having serial assays performed. Because the assay is not designed to be accurate at the levels encountered in this phase of pregnancy it is only possible to consider the tentative limits for the lower end of the "favourable" range and these are shown in Table III. Out of 11 pregnancies with "favourable" values only 2 had abortions soon after the sample of blood had been obtained but 3 of the remaining 9

TABLE III

*HPL levels before 9 weeks gestation*

| Week of gestation | Lower limit of favourable zone (µg./ml.) |
|---|---|
| 7 | 0·006 |
| 7½ | 0·010 |
| 8 | 0·015 |
| 8½ | 0·021 |

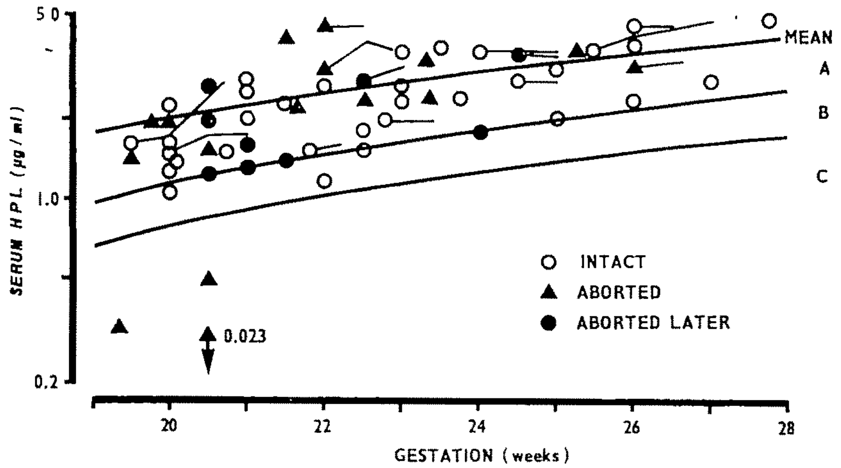

FIG. 6

Single and serial HPL levels in threatened abortions after 19 weeks gestation.

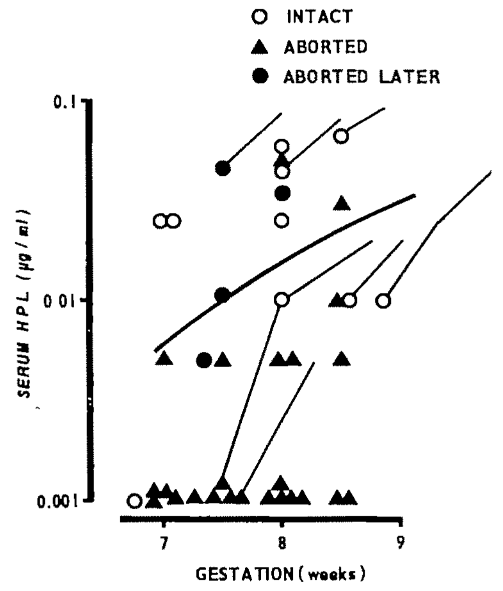

FIG 7
Single and serial HPL levels in threatened abortion before the 9 weeks gestation. The thick line represents the tentative limits of the "favourable" zone.

intact pregnancies subsequently aborted. Of the 27 pregnancies in which the result lay below the favourable value only five had viable pregnancies. All data for this range of pregnancy are shown in Figure 7.

In 27 patients in whom pregnancy was suspected but confirmatory evidence lacking, the HPL level was always in the "unfavourable" range.

## DISCUSSION

The present study is the largest to date to evaluate HPL as an index of viability in the first two trimesters of pregnancy.

Support is given to previous claims (Ylikorkala and Jouppila, 1973) that normal and abnormal HPL levels are related to the method of assay. Using the Radiochemical Centre's kit the results in this study correlated more closely with those obtained by Niven *et al.*, 1972. The value of the HPL level between the 9th and 19th week seems indisputable.

The adoption of a scheme for assigning an HPL result into a "favourable", "equivocal" or "unfavourable" category would appear to offer the clinician the maximum value from the assay. The importance of the period of gestation cannot be overemphasized particularly when considering borderline "favourable" or "unfavourable" results. In these cases the difference of just over a week could result in a wrong prognosis.

Whilst the necessity for serial assays is disputed when either a satisfactory "favourable" or "unfavourable" result has been obtained it is accepted that repeat tests may occasionally be of value.

That considerable time is spent in hospital prior to abortion or evacuation has already been shown. Many of the patients in whom a pregnancy eventually proved non-viable awaited the results of two or even three negative pregnancy tests performed on successive days prior to evacuation. There is naturally a reluctance to evacuate a pregnancy which may prove to have been normal on the evidence provided by one laboratory test; the extra information provided by an HPL level could therefore be invaluable. For this test only a serum sample is required avoiding the necessity of collecting a 24-hour sample of urine for other quantitative hormone assays.

It is not really surprising that HPL levels are of little value in late second trimester abortion as many of these represent "premature labour" with a normally functioning placenta with abortion taking place for reasons other than fetal and placental failure.

## ACKNOWLEDGEMENTS

We are indebted to the medical and nursing staff at the participating hospitals for their co-operation, Dr. R. J. Bayly for the statistical analysis and the Radiochemical Centre for providing the material used for the assays.

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# A REVIEW OF 700 HYSTEROTOMIES

BY

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## Summary

We studied 700 hysterotomies carried out in Aberdeen between 1968 and 1972. The morbidity and mortality indicate that the operation is now outdated as a method of terminating pregnancy. A study of referral practice showed that there were many unnecessary delays.

SEVEN hundred cases of hysterotomy, 582 (83 per cent) of which were combined with sterilization, have been reviewed in terms of immediate morbidity and mortality with special emphasis on the problem of thromboembolic complications. As many as possible of those patients who had a hysterotomy alone were followed in order to assess their subsequent reproductive performance.

The available literature on this operation is scanty. Stallworthy *et al.* (1971), reviewing 70 cases, remarked on the unacceptably high long- and short-term morbidity. Tietze and Guttmacher (1973) found the mortality of the operation (208·3 per 100 000) to be much higher than that from suction aspiration, dilation and curettage, or intra-amniotic saline. Clow and Crompton (1973), in a review of 45 patients who between them had 53 pregnancies after a hysterotomy found evidence of a substantial risk of rupture of the uterus and a high incidence of small-for-dates infants.

In most centres in Britain this operation is being replaced by what are considered better methods of second trimester abortion using prostaglandins and in a recent American review (Burnett *et al.*, 1974), hysterotomy is not even mentioned.

## RESULTS

During the five-year period of the study, 700 out of 2891 terminations were by hysterotomy.

### *Age distribution*

Of the patients having hysterotomy alone, 65 per cent were under the age of 25, whereas 90 per cent of those having hysterotomy and sterilization were over the age of 25.

### *Marital status*

Most patients having a hysterotomy alone were unmarried and 11 single women were sterilized either because they had had a number of previous illegitimate pregnancies (three patients) or because they were in psychiatric care. Twenty-six patients who had a hysterotomy alone were separated from their husbands or divorced.

### *Use of anticoagulants*

In 1968 only 9 per cent of patients had prophylactic anticoagulants, but in 1972 the figure was 60 per cent. Warfarin was used in 1968 to 1970 except for two patients who were given phenindione. After Dextran 70 was introduced in 1971, Warfarin was used only occasionally. In all, 15·7 per cent of patients

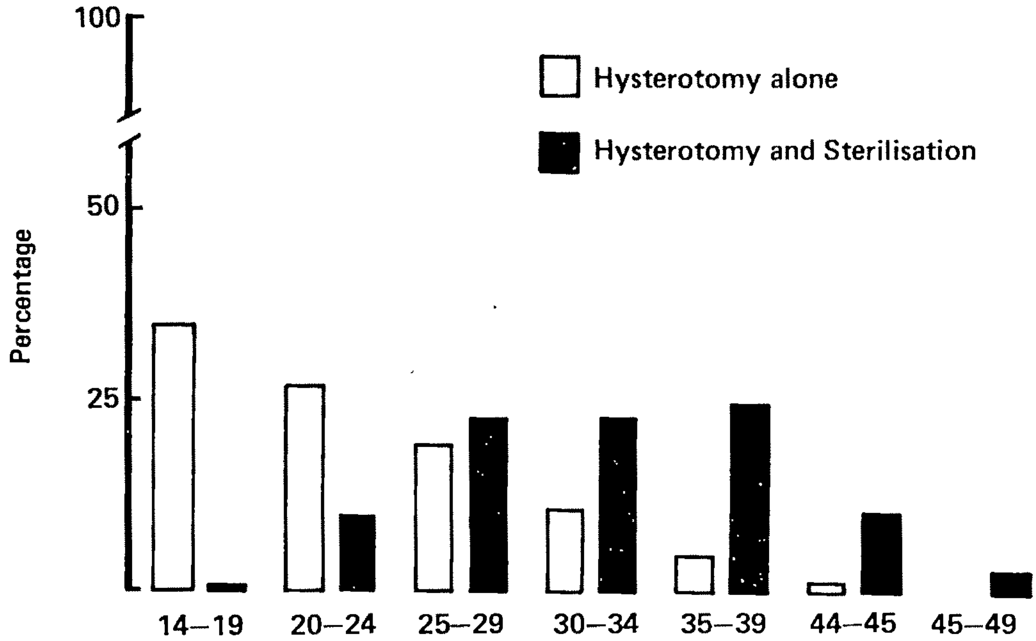

FIG. 1

Age group distribution for patients having a hysterotomy between 1968 and 1972.

had Dextran 70 during the operation only and a further 7·7 per cent had Dextran 70 both in the operating theatre and on two days thereafter.

*Thromboembolic complications*

There were 21 patients (3 per cent) with thromboembolism (Table I); thirteen had a pulmonary embolus of which three had an iliofemoral thrombosis treated by thrombectomy. Half of these patients had anticoagulants.

There was one death from massive pulmonary embolism in a multiparous patient aged 30 who had received no anticoagulant prophylactically despite the presence of marked varicose veins.

TABLE I

*Patients with thromboembolism*

| *Thromboembolism (21 patients)* | |
|---|---|
| Superficial thrombophlebitis | 1 |
| Deep venous thrombosis | 7 |
| Pulmonary infarction | 9 |
| Iliofemoral thrombosis and pulmonary infarction | 3 |
| Massive pulmonary embolism and death | 1 |

No other deaths occurred in 2891 terminations of pregnancy done between 1968 and 1972.

*Effect of sterilization*

The addition of sterilization to the operation increased the risk of thromboembolism fourfold but the patients being sterilized were older, more obese, and of higher parity and therefore more likely victims. The majority of patients with thromboembolism were over 25 years of age and the incidence of thromboembolism in the over 25-year-old group was 3·4 per cent compared with 1·4 per cent for those under 25.

*Previous history*

While approximately one-third of all patients received anticoagulants, two-thirds of those with a previous history of thromboembolism did so. The incidence of thromboembolism was 4 out of 30 (13·3 per cent) for patients with a previous history of thromboembolism as against 17 out of 670 (2·6 per cent) for a patient with no such history.

TABLE II

*Complications other than thromboembolism*

| | No. | Per cent |
|---|---|---|
| Urinary tract infection | 68 | 9·7 |
| Respiratory infection | 38 | 5·4 |
| Wound infection | 20 | 2·8 |
| Wound dehiscence | 12 | 1·7 |
| Pyrexia of unknown origin | 16 | 2·2 |
| Haemorrhage | 1 | 0·1 |
| Ileus | 1 | 0·1 |
| Erythema nodosum | 1 | 0·1 |
| Pelvic infection | 1 | 0·1 |

### *Other complications*

The other complications which occurred are listed in Table II. It should be noted that almost 80 per cent of patients had no immediate complications. Many of the patients who had pyrexia of unknown origin probably had a genital tract infection.

### *Referral practice*

An analysis of referral practice is shown in Table III. Of 501 patients about whom the relevant information was available, 80 per cent were seen by a general practitioner before 12 weeks and 90 per cent before 14 weeks. Further, 61·5 per cent of the 501 patients were at less than 12 weeks gestation when seen at the gynaecological outpatient clinic and 81·8 per cent were at less than 14 weeks gestation. However, by the time patients were admitted only 17·3 per cent were at less than 12 weeks gestation and 42·7 per cent less than 14 weeks. There were 38 patients who had their operation after 20 weeks gestation, two as late as 27 weeks.

TABLE III

*Referral practice in patients having a hysterotomy in Aberdeen 1968 to 1972*

| | Delay between dates of general practitioner's letter and attendance at the gynaecological clinic | | Delay between outpatient consultation and admission | |
|---|---|---|---|---|
| | No. | Per cent | No. | Per cent |
| 7 days | 82 | 16·4 | 187 | 30·3 |
| 8–14 days | 190 | 37·9 | | |
| 15–21 days | 174 | 34·7 | 300 | 48·6 |
| 22–28 days | 40 | 8·0 | | |
| Greater than 28 days | 15 | 3·0 | 130 | 21·1 |
| Total | 501 | 100 | 617 | 100 |

The interval between outpatient consultation and admission was long, but perhaps this was because there seemed no need for hurry in patients down for hysterotomy. Another factor in the early years was the practice of referral to psychiatrists for an opinion. In 1968 over 57 per cent of patients were admitted more than three weeks after the decision to terminate had been made. In 1972 that figure was still 30 per cent with 62 per cent being admitted after two weeks.

### *Subsequent pregnancies*

Twenty-six of our patients who had a hysterotomy had a total of 30 pregnancies subsequently and there was one set of twins. There were no spontaneous abortions or further terminations. There were 27 vaginal deliveries, three emergency Caesarean sections and one elective Caesarean section. Scar rupture did not occur and only 2 out of 31 babies had a birthweight below the tenth centile (Thomson *et al.*, 1968).

## DISCUSSION

Although our death rate of 1 in 700 compares favourably with the 5 in 2400 (1 in 480) found by Tietze and Guttmacher (1973), it is about ten times the mortality risk of pregnancy in Scotland and also compares very unfavourably with other methods of termination of pregnancy. Four of six deaths after termination of pregnancy in Wales between 1969 and 1972 followed hysterotomy (Jones and Jones, 1973). This review covered 11 680 terminations of pregnancy of which 3099 were by hysterotomy. Although our numbers were small, we found no evidence of hysterotomy scar weakness which has been reported by others (Russell and Hewlett, 1969; Clow and Crompton, 1973).

Our morbidity due to infection and thromboembolism was significant. Whether prophylactic anticoagulation prevents thromboembolism is uncertain. Perhaps the most disquieting feature of all was the delay between consultation with the general practitioner and hospital admission. If patients had been seen within three to four days of visiting their general practitioner, over 80 per cent could have had a vaginal termination. It is also noteworthy that despite the delays 40 per cent of the hysterotomies were performed at less than 14 weeks gestation when pregnancy could have been terminated by suction. In some instances, simultaneous sterilization was the reason for choosing hysterotomy and the introduction of laparoscopic sterilization has removed the need for such a practice.

In Aberdeen the number of hysterotomies have fortunately fallen from 188 in 1971 to 34 in 1973. With further streamlining and improvement of referral practice hysterotomies and most mid-trimester abortions should become things of the past.

## ACKNOWLEDGEMENTS

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# ELECTROENCEPHALOGRAPHIC CHANGES AFTER INTRA-AMNIOTIC PROSTAGLANDIN $F_{2\alpha}$ AND HYPERTONIC SALINE

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## Summary

Six major convulsions have been seen among 555 patients who had therapeutic second trimester abortion by intra-amniotic prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Five of 21 patients receiving intra-amniotic hypertonic saline and 5 of 16 patients receiving $PGF_{2\alpha}$ showed abnormalities in electroencephalograms after treatment. These changes were relatively minor in all saline patients and in three receiving $PGF_{2\alpha}$, but two patients receiving $PGF_{2\alpha}$ showed epileptiform spike activity after infusion. There was no correlation between the electroencephalographic (EEG) changes and peripheral venous levels of $PGF_{2\alpha}$ or its metabolite, 13, 14 dihydro-15 keto $PGF_{2\alpha}$ (15 keto-$h_2$-$F_{2\alpha}$).

A PREVIOUS study from this centre described five patients with major convulsions and four others with induced electroencephalographic (EEG) changes in a group of women who had therapeutic second trimester abortion by intra-amniotic prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Lyneham *et al.*, 1973). While some investigators have not noted similar EEG changes after administration of either $PGF_{2\alpha}$ or $PGE_2$ (MacKenzie *et al.*, 1973; Fraser and Gray, 1974; Thiery *et al.*, 1974) in small numbers of patients, Van Der Plaetsen and others (1974) noted deterioration in an already abnormal EEG after administration of $PGE_2$ intra-amniotically.

We have now seen six patients with major convulsions among 555 pregnancies terminated with intra-amniotic $PGF_{2\alpha}$. The EEG study has been extended to a series of 16 women having $PGF_{2\alpha}$ and 21 women receiving intra-amniotic hypertonic saline. Peripheral venous blood was obtained from 15 patients for assay of $PGF_{2\alpha}$ and its metabolite 15 keto-$h_2$-$F_{2\alpha}$.

## MATERIALS AND METHODS

### *Patients*

Eight patients received 30 mg. of $PGF_{2\alpha}$ via an intra-amniotic catheter; if abortion did not occur within 24 hours, a further 15 mg. was given and if necessary, repeated 18 hours later. The results in this group have been reported (Lyneham *et al.*, 1973). A further eight patients received 40 mg. of $PGF_{2\alpha}$ via a Tuohy needle which was removed immediately. Twenty-one patients had intra-amniotic administration of 150 to 200 ml. of 20 per cent saline after aspiration of a similar quantity of amniotic fluid.

All patients were 16 to 20 weeks pregnant. None had a history of previous epilepsy. Additional therapy was restricted rigidly to pethidine for pain and metoclopramide for nausea.

### *EEG recordings*

Sixteen or eight-channel EEG recordings were performed on all patients prior to treatment and at both one and four hours after commencement, using 21 surface electrodes applied in accordance with the International 10/20 system. The recordings were reported without knowledge of the name of the patient, the method of inducing abortion or the sequence of the recording.

### *Radioimmunoassay of $PGF_{2\alpha}$ and 15 keto-$h_2$-$F_{2\alpha}$*

Peripheral venous blood was withdrawn during the interval of each EEG recording from seven patients receiving hypertonic saline and eight patients receiving 40 mg of $PGF_{2\alpha}$.

Antisera to $PGF_{2\alpha}$ and its metabolite, 15 keto-$h_2$-$F_{2\alpha}$, were raised in rabbits as described by Caldwell *et al.* (1971). The method of preparation of the tritium labelled metabolite and that of the double antibody radioimmunoassay for $PGF_{2\alpha}$ and its metabolite were essentially as described by Granstrom and Samuelsson (1972) and Cornette *et al.* (1974). Cross reaction of the $PGF_{2\alpha}$ antibody with 15 keto $PGF_{2\alpha}$ or 15 keto-$h_2$-$F_{2\alpha}$ was less than 0·1 per cent and cross reaction of the 15 keto-$h_2$-$F_{2\alpha}$ antibody with $PGF_{2\alpha}$ or 15 keto $PGF_{2\alpha}$ was less than 1 per cent.

## RESULTS

### *Radioimmunoassays*

The results are given in Table I. The seven patients receiving hypertonic saline showed no

TABLE I

*$PGF_{2\alpha}$ and 13, 14 dihydro-15 keto $PGF_{2\alpha}$ concentrations in peripheral venous plasma*

| Treatment and Patient No. | Plasma concentration (ng/ml) | | | | | |
|---|---|---|---|---|---|---|
| | Pre-treatment | | After 1 hour | | After 4 hours | |
| | $PGF_{2\alpha}$ | 15 keto-$h_2$-$F_{2\alpha}$ | $PGF_{2\alpha}$ | 15 keto-$h_2$-$F_{2\alpha}$ | $PGF_{2\alpha}$ | 15 keto-$h_2$-$F_{2\alpha}$ |
| Intra-amniotic saline | | | | | | |
| 1* | 0·32 | 5·0 | 0·13 | 5·5 | 0·25 | 6·5 |
| 2 | 0·50 | 6·5 | 0·50 | 6·0 | 0·56 | 7·5 |
| 3* | 1·50 | 7·5 | 0·50 | 4·0 | 1·38 | 10·6 |
| 4 | 1·13 | 7·3 | 0·63 | 6·3 | 0·75 | 8·8 |
| 5 | 1·00 | 8·1 | 0·75 | 4·9 | 0·50 | 5·5 |
| 6 | 1·13 | 9·0 | 0·63 | 8·8 | 0·88 | 11·6 |
| 7 | 0·50 | 5·9 | 0·50 | 6·6 | 0·75 | 9·5 |
| Mean | 0·87 | 7·0 | 0·52 | 6·0 | 0·72 | 8·6 |
| ± SE | 0·16 | 0·5 | 0·07 | 0·6 | 0·13 | 0·8 |
| Intra-amniotic $PGF_{2\alpha}$ | | | | | | |
| 1 | 0·38 | 5·5 | 0·44 | 25 | 0·50 | 24 |
| 2* | 0·38 | 5·3 | 0·38 | 25 | 0·56 | 23 |
| 3† | 0·10 | 6·0 | 7·00 | 65 | 0·60 | 16 |
| 4 | 1·13 | 7·5 | 1·00 | 38 | 0·50 | 44 |
| 5 | 0·38 | 5·4 | 0·50 | 18 | 0·63 | 20 |
| 6 | 0·25 | 7·8 | 0·50 | 19 | 0·25 | 21 |
| 7 | 0·50 | 6·6 | 1·13 | 43 | 0·75 | 23 |
| 8 | 0·25 | 5·8 | 1·25 | 103 | 0·25 | 9 |
| Mean | 0·42 | 6·2 | 1·53 | 42 | 0·51 | 23 |
| ± SE | 0·11 | 0·3 | 0·79 | 10 | 0·06 | 4 |

*Abnormal EEG.
†Acute reaction.

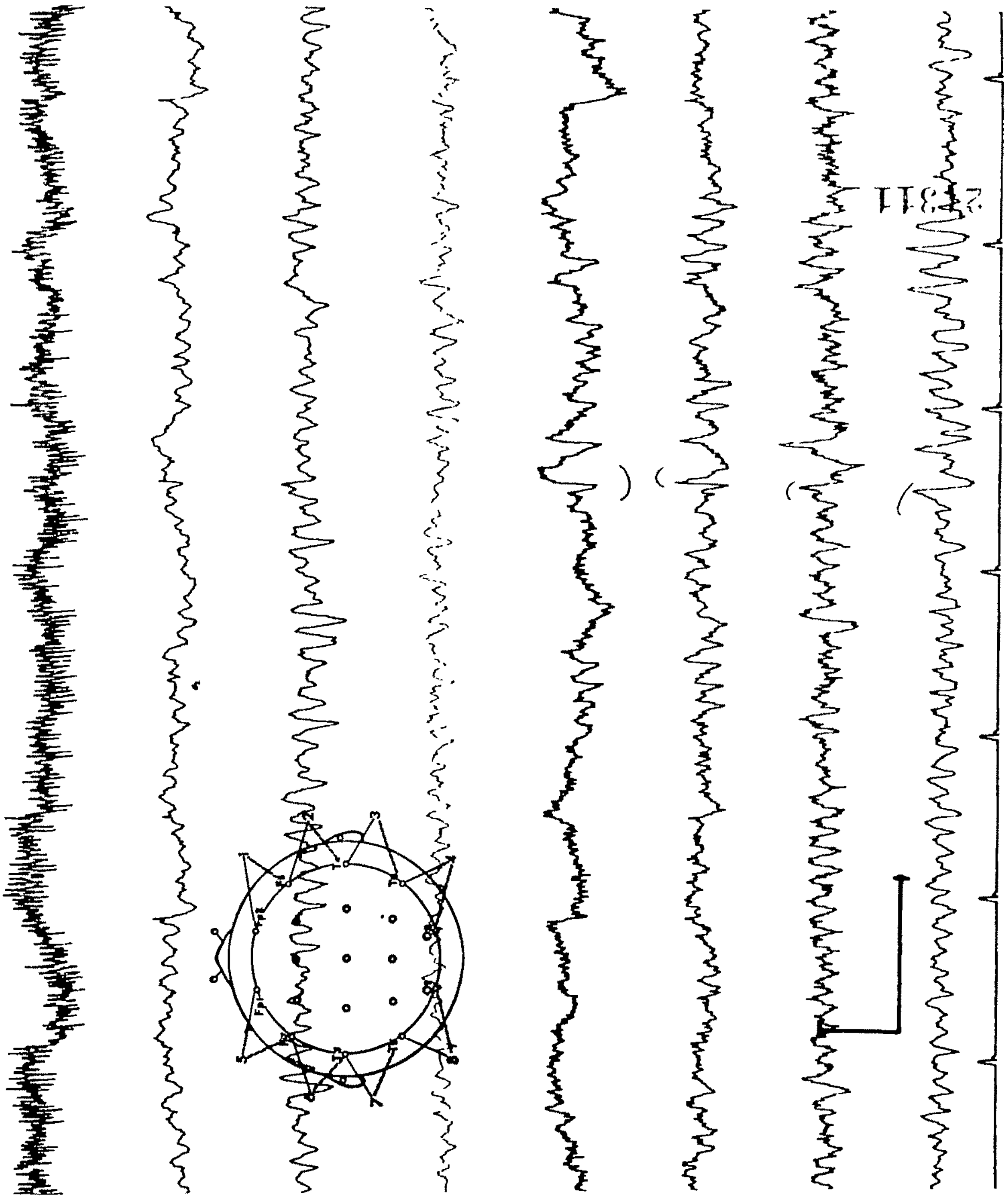

FIG. 1

Portion of EEG recording from patient No. 2, one hour after instillation of PGF$_{2\alpha}$. Focal phase reversed sharp waves are seen in the left temporal area. Scale: one second and 150 micro volts.

changes in PGF$_{2\alpha}$ or 15 keto-h$_2$-F$_{2\alpha}$ levels in peripheral venous plasma.

The eight patients receiving PGF$_{2\alpha}$ showed, with one exception, no increase in venous levels of PGF$_{2\alpha}$; levels of the metabolite were grossly increased at one and four hours. One patient in this group (Patient No. 3) had an amniotomy yielding blood-stained fluid. One minute after infusion of PGF$_{2\alpha}$ she was anxious, sweating, flushed, dyspoenic and vomited. Although the clinical symptoms subsided within ten minutes, PGF$_{2\alpha}$ levels were still considerably elevated at one hour but not at four hours.

### *EEG*

Of the 21 patients receiving saline, five had minor phase-reversed theta transients over the temporal areas before infusion; none of these changed after induction. The remaining 16 records were normal initially; in this group five records contained asymmetrical theta activity with phase reversal in the temporal areas following induction. No spike activity occurred in any of the patients in the saline group.

The pretreatment tracing in the 16 patients receiving PGF$_{2\alpha}$ was normal or showed insignificant amounts of 4 to 7 Hz theta activity. Eleven patients showed no changes after induction. Three patients showed relatively minor changes after induction, right fronto-parietal delta activity in one patient at four hours, generalized theta slow waves with right-sided emphasis in another at four hours and 4 to 6 and 2 to 3 Hz slow wave activity over the left temporoparietal region and a left fronto-temporal sharp wave focus at both one and four hours in the third. Two patients showed major spike discharges at both one and four hours. In one of these, the discharges were seen over both hemispheres with anterior temporal emphasis; in the other (Patient No. 2) there were phase reversed sharp wave discharges in the left temporal area as shown in Figure 1.

## DISCUSSION

Changes in the EEG were noted in 5 of 21 patients after receiving intra-amniotic hypertonic saline and 5 of 16 patients receiving intra-amniotic PGF$_{2\alpha}$. It seems therefore that some EEG changes are relatively common during induced second trimester abortion, irrespective of the method used. However, no patient in the saline group showed spike activity while two women in the PGF$_{2\alpha}$ group showed clear epileptiform spike activity after infusion.

There appears to be no relationship between induction of EEG abnormalities and systemic levels of either PGF$_{2\alpha}$ or one of its major metabolites, or at least as measured in peripheral blood. We do not have any information on levels in cerebrospinal fluid. It should be noted that the patient who developed a marked systemic reaction with very high venous levels of PGF$_{2\alpha}$ one hour after infusion showed no change in the EEG.

These findings indicate that two patients in the prostaglandin group and none in the saline group had evidence of definite epileptiform spike activity following treatment. Until the position is clarified further, we will avoid using intra-amniotic PGF$_{2\alpha}$ for termination of second trimester pregnancy in patients with a history of epilepsy.

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# OPERATIVE CULDOSCOPY

BY

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## Summary

A study of 257 operative culdoscopies done without general anaesthesia is presented and the advantages and limitations of the technique are detailed.

ALTHOUGH recently overshadowed by the popularization of laparoscopic techniques, we believe that there is still a place for operative culdoscopy especially as it can be performed without general anaesthesia.

Decker (1952) developed the culdoscope and was successful in performing through it some minor surgery on the pelvic organs. Clyman (1963) attached surgical tools and with this "panculdoscope" operated on patients in the knee-chest position, separating adhesions and taking biopsies of the ovaries and aspirating ova for physiological studies. Later, Clyman (1968) found it less restrictive to abandon fixing the tools to the shaft of the optic in favour of passing surgical instruments alongside the culdoscope. In this way one can operate inside the pelvis or bring the tubes or ovaries into the vagina for operation under direct vision.

## TECHNIQUE

The patient is admitted the day prior to surgery. She takes ampicillin orally daily and inserts a nifurantoin vaginal pessary daily for seven days starting two days before operation.

One-and-a-half hours preoperatively, the patient receives 4 mg. of droperidol, 0·5 mg. of scopolamine and 20 mg. of papaveretum intramuscularly, and ten minutes before the operation 50 mg. of pethidine intravenously. During the operation 5 to 10 mg. of diazepam is added intravenously if required.

The patient positions herself in the knee-chest position on the flat operating table which has two shoulder braces to prevent her from slipping forwards. Her head is turned to the left, resting on the table between the braces.

After insertion of a Clyman (1968) self-retaining vaginal speculum, paracervical block with 2 ml. of 1 per cent lignocaine with adrenaline 1 : 400 000 on each side is performed and 1 ml. may also be infiltrated into the centre of the posterior fornix.

The procedure begins as a routine diagnostic culdoscopy. If pathology is seen which can be treated by this route the vaginal skin is infiltrated once more around the culdoscope which is then removed. The culdoscope incision is then widened laterally and the operation performed *in situ* or after delivering the tube or ovary into the vagina with a special soft clamp (Clyman, 1968). After completion of the procedure, a single figure-of-eight suture (chromic catgut No. 00) is inserted which includes both the

TABLE I

*Indications for culdoscopy*

| | |
|---|---|
| Primary sterility | 101 |
| Secondary sterility | 79 |
| Sterilization | 36 |
| Abdominal-pelvic pain | 10 |
| Ovarian biopsy | 8 |
| Suspected ovarian swelling | 8 |
| For oophorectomy for breast cancer | 7 |
| Suspected ectopic pregnancy | 6 |
| Removal of fimbrial prosthesis | 2 |
| | 257 |

peritoneum and the vaginal skin with each bite. Before this suture is tied the culdoscope cannula is reinserted and the patient placed in the prone position on the surgeon's hand to expel the air from the peritoneal cavity. The cannula is then removed and the suture tied. The patient leaves hospital the next day.

## RESULTS

Between the years 1968 to 1973, 257 operative culdoscopies were attempted: in 180 patients for infertility, in 36 for sterilization and in 41 for various other indications (Table I).

In 18 out of these 257 patients (6·5 per cent) we failed to penetrate the pouch of Douglas; in most cases subsequent laparotomy showed that this was because of pelvic adhesions. In a further 33, the diagnostic culdoscopy revealed pathological findings which could not be treated by this route. In 55 women there were no abnormal findings so no operative procedure was required.

In the remaining 151 patients 237 procedures were performed (Table II). Lysis of adhesions was done mainly in cases of infertility where slight adhesions around the ovary and tube were found. Any consequent bleeding points were electrocauterized. Because of the danger of bleeding we did not use lysis by this route when adhesions were dense and thick.

TABLE II

*Surgical procedures performed*

| | |
|---|---|
| Dilatation of fimbrial end of tube | 65 |
| Tubal sterilization | 62 |
| Wedge resection of ovaries | 31 |
| Division of adhesions | 29 |
| Ovarian biopsy | 15 |
| Oophorectomy | 13 |
| Puncture of cystic ovary | 8 |
| Salpingectomy | 5 |
| Ovarian cystectomy | 4 |
| Removal of fimbrial prosthesis | 2 |
| Coagulation of endometriosis | 2 |
| Salpingectomy for tubal pregnancy | 1 |
| | 237 |

There were 71 procedures on the ovary and the simplicity of their performance is one of the major advantages of operative culdoscopy. Ovarian biopsy, puncture of ovarian cysts and cauterization of ovarian endometriosis were performed with the ovary in normal position. Wedge resection, oophorectomy and cystectomy were performed after delivering the ovary into the vagina. Cystectomy may be performed if the diameter of the cyst is not more than 4 to 5 cm. Difficulty in delivering an intact cyst into the vagina may be overcome by the prior aspiration of its fluid contents. We have found this route particularly advantageous for wedge resection of polycystic ovaries (Paldi *et al.*, 1972), and for bilateral oopherectomy in cases of breast cancer.

We advocate tubal sterilization through culdoscopy rather than laparoscopy because general anaesthesia can be avoided and the procedure is done under direct vision. In cases where passage through the fimbriated end of the tube of dye instilled through the cervix is delayed or restricted, the outer end of the tube can be delivered into the vagina, dilatation performed under direct vision and free spillage of the dye observed. Reconstructive tubal surgery by this route is limited to when the fimbriated end is occluded in a free and mobile tube. We performed salpingostomy in five cases only, but removal of a fimbrial prosthesis (silastic cap of Mulligan) through operative culdoscopy saves a second laparotomy.

We performed salpingectomy in one case of tubal pregnancy diagnosed by culdoscopy (Timor-Tritsch *et al.*, 1974). As the tube was unruptured and mobile with the conceptus in the ampulla and without free blood in the pelvic cavity, salping-ectomy was done after delivering the tube into the vagina. Such circumstances are, however, exceptional.

### *Complications*

In two patients early in this series the rectum was perforated but repair was uneventfully successful, and in one of them we performed the culdoscopy successfully two months later.

There were three pelvic infections, all after tubal sterilizations. Two responded to additional antibiotic treatment but the third required surgical drainage of a tubovarian abscess.

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# THE INVESTIGATION OF OVARIAN FUNCTION BY MEASUREMENT OF URINARY OESTROGEN AND PREGNANEDIOL EXCRETION

BY

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## Summary

Urinary oestrogen and pregnanediol excretion was measured daily ("daily monitoring") for a complete cycle in 20 normally menstruating women, in one patient with an anovulatory cycle and for 28 days in a patient with secondary amenorrhoea. The measurements were also performed on urine specimens collected at weekly intervals for 4 to 6 weeks ("weekly tracking") from 506 patients with evidence of abnormal ovarian function. These included 9 patients with primary amenorrhoea, 132 patients with secondary amenorrhoea, 138 patients with oligomenorrhoea and 227 patients with evidence of ovarian dysfunction and cycle lengths of 25 to 42 days. The results were subjected to statistical analysis. In the normal cycles, ovulation could be identified on the criteria of a rising pregnanediol value reaching or exceeding 2·0 mg. per 24 hours for a period of 7 days or more. Valid conclusions on the overall mean oestrogen and pregnanediol values for a complete cycle could be made from the results of weekly tracking, irrespective of which day the tracking commenced. Correlations were obtained by comparing the mean and maximum urinary oestrogen values and the variability of the values with the evidence of ovarian function indicated by the clinical classifications of the patients, the duration of the disorders and the subsequent occurrence of uterine bleeding. Mean oestrogen values of 10 $\mu$g. per 24 hours or less were associated with lack of ovarian function. For values higher than this a discriminant function based on both the mean oestrogen value and the variability of the oestrogen values was useful in predicting onset of spontaneous menstruation. A single urine specimen collected 4 to 8 days before onset of menstruation showing a raised pregnanediol value of 2·0 mg. per 24 hours or more provided a valid test for ovulation in women with regular cycles, and a single urine specimen giving an oestrogen value of 10 $\mu$g. per 24 hours or less gave a valid indication of absent ovarian function in women with amenorrhoea for two years or more. In all other circumstances serial sampling at weekly intervals provided a valid assessment of ovarian activity. Application of these principles allows the greatest amount of information on ovarian function to be obtained with the greatest economy of effort.

IN the treatment of disorders of menstruation, particularly when infertility is the problem, tests of ovarian and pituitary function are most useful for assessing the nature and the permanency of the disorder. Measurement of ovarian and pituitary hormones in blood or of their metabolites in urine has been widely used for this purpose and because ovarian activity is cyclic, the need for multiple determinations over considerable periods of time, has been repeatedly stressed. Although determinations performed daily over at least one cycle of ovarian activity provide the maximum information, this is clearly too laborious to be applied to the assessment of large numbers of patients. Therefore, a procedure involving oestrogen and pregnanediol measurements on specimens of urine collected weekly has been investigated. The present paper reports the findings in various categories of anovulatory patients attending the Endocrine and Infertility Clinics of the Royal Women's Hospital, Melbourne. The results are compared with those obtained from normally ovulating women, and the information provided by the weekly measurements is compared with that provided by daily measurements on the one hand and by a single timed measurement on the other. The results are correlated with clinical assessments of the patient and with the duration of amenorrhoea and the subsequent onset of spontaneous menstruation.

## MATERIALS AND METHODS

### *Methods*

Total urinary oestrogens were measured by the method of Brown *et al.* (1968) utilizing a mechanical extractor and fluorimetry. The coefficient of variation of the method was 6 per cent at excretion rates of less than 10 $\mu$g. per 24 hours and 4 per cent at higher rates. Interassay variation of a control urine performed with every assay was 8·2 per cent (n = 100).

Urinary pregnanediol was measured by gas-liquid chromatography as described by Cox (1963). The sensitivity of the method was 0·05 mg. per 24 hour urine and the coefficient of variation was 3 per cent at excretion rates of less than 1 mg. per 24 hours, and 2.5 per cent at higher rates. The interassay variation of the same urine specimen analyzed in 15 consecutive assays was 4·9 per cent (Barrett and Brown, 1970).

Urinary luteinizing hormone (LH) was extracted by a modification of the method of Baghdassarian *et al.* (1970) and measured by a specific double antibody radioimmunoassay as described by Alford *et al.* (1973). The coefficient of variation was less than 6 per cent for values in the range seen in young adult men and women. Interassay variation of the same urine specimen analyzed in 20 consecutive assays was 9·6 per cent. This test provided additional evidence that ovulation occurred in the control group of women who collected daily specimens.

### *Collection of urine specimens*

*Daily collections* ("*daily monitoring*"). Twenty parous women ("normal controls") with apparently normal menstrual cycles collected daily urine specimens throughout one complete cycle. The urine specimens were analyzed for total oestrogens, pregnanediol and LH. Two other women who were anovulatory during the period of study also collected daily urine specimens for 4 weeks.

*Weekly collections* ("*weekly tracking*"). As part of their routine investigation, 506 patients (Table III) attending the Endocrine and Infertility Clinics of the Royal Women's Hospital collected 24-hour specimens of urine at weekly intervals for 4 to 6 weeks. The reason for their attendance was infertility associated with some disorder of menstruation. Nine of these patients had primary amenorrhoea, 132 had secondary amenorrhoea defined as absence of menses for 6 months or more, 138 had oligomenorrhoea, defined as cycle lengths of 6 weeks to 6 months, and 227 had cycles lasting for 24 to 42 days. This latter group of 227 patients comprised patients with some evidence in preceding cycles of abnormal ovarian function which might have affected their fertility such as the finding of a monophasic temperature chart or poor secretory changes in the endometrium; they were studied from one bleeding episode to the next.

### *Storage of urine specimens*

Oestrogen and pregnanediol measurements were usually performed on the same day that the urine was received or sometimes after storage at 4 °C. for a maximum of three days. Specimens for the measurement of LH were stored at −20 °C. and the specimens from one patient were all measured in duplicate in the same assay.

### *Statistical analysis*

A computer programme was designed to provide the following data relating to oestrogen and pregnanediol excretion for each woman:

(*a*) the maximum value obtained in the serial sampling,
(*b*) the mean value for the series, and
(*c*) the standard deviation (SD) of the mean, which provided an assessment of the fluctuation in values during the period of study.

These data were correlated with the presence or absence of ovulation, with subsequent spontaneous menstruation and with clinical particulars such as age, weight, hirsuties, galactorrhoea, and the taking of oral contraceptives.

## RESULTS

### *Normal subjects (daily urine collections)*

All of the 20 normal women who provided daily urine collections ovulated during the cycle studied as judged by the finding of mid-cycle peaks of urinary oestrogen and LH excretion followed by a rise in pregnanediol excretion. An assessment of the pregnanediol values in these cycles was important because they provided the basis for stating whether ovulation occurred in the patients during weekly tracking. The maximum pregnanediol value found in an individual ranged from 2·5 mg. to 7·7 mg. per 24 hours. In 19 out of the 20 subjects, the pregnanediol values were equal to or greater than 2·0 mg. per 24 hours for at least seven consecutive days during the luteal phase and in the remaining subject they were maintained at these figures for five days. Because of these findings, urinary pregnanediol values rising to 2·0 mg. per 24 hours or more have been accepted as evidence of ovulation in this study. On this basis, 19 of the normal cycles would have been considered ovulatory, regardless of which of the first seven days of the cycle was used as the first day for commencing weekly tracking, and an error in interpretation of the remaining normal cycle would have been made if tracking had commenced on two of the first seven days.

A correct assessment of the ovulatory nature of the cycles could have been made on the basis of one urine specimen only, providing that the collection was made between 4 and 8 days inclusive prior to the onset of menstruation. Extending this time interval to three to nine days would have led to an error in interpretation in one cycle.

The mean values for oestrogen and pregnanediol excretion throughout each cycle were calculated from all of the daily values and from each of the seven weekly combinations (four values each). The results from one of the normal subjects are shown in Table I. In the total series of 20 normal cycles, no significant difference was demonstrated between the mean values calculated from all of the daily urine specimens and those calculated from weekly tracking commen-

TABLE I

*Mean urinary oestrogen and pregnanediol values calculated from daily and weekly estimations during an ovulatory cycle, an anovulatory cycle and a period of ovarian inactivity in three different subjects*

| | Oestrogen excretion (µg./24 hours) Mean±SD | | | Pregnanediol excretion (mg./24 hours) Mean±SD | | |
|---|---|---|---|---|---|---|
| | Ovulatory | Anovulatory | Amenorrhoea | Ovulatory | Anovulatory | Amenorrhoea |
| Complete daily values | 15·9±9·0 | 8·7±6·6 | 4·2±1·4 | 1·2±1·1 | 0·14±0·08 | 0·19±0·09 |
| Weekly values on: | | | | | | |
| Days 1, 8, 15, 22. | 17·2±14·7 | 4·9±3·9 | 3·8±1·2 | 1·1±1·2 | 0·13±0·04 | 0·23±0·07 |
| 2, 9, 16, 23. | 15·9±10·8 | 8·2±3·0 | 3·5±0·5 | 1·4±1·6 | 0·10 | 0·18±0·08 |
| 3, 10, 17, 24. | 14·1±5·6 | 9·7±6·6 | 3·0±0·6 | 1·0±0·7 | 0·15±0·05 | 0·18±0·08 |
| 4, 11, 18, 25. | 13·6±6·1 | 10·2±5·5 | 5·4±1·2 | 1·3±1·1 | 0·10 | 0·20±0·00 |
| 5, 12, 19, 26. | 15·2±7·4 | 9·0±6·6 | 5·1±1·1 | 1·3±0·9 | 0·23±0·16 | 0·18±0·04 |
| 6, 13, 20, 27. | 18·6±4·2 | 8·8±9·4 | 4·9±1·8 | 1·3±0·7 | 0·15±0·05 | 0·20±0·07 |
| 7, 14, 21, 28. | 16·7±8·9 | 10·3±7·3 | 3·7±0·9 | 1·2±1·1 | 0·13±0·04 | 0·15±0·09 |

cing on any of the first seven days of the cycles. However, the means found in individual cycles ranged from 11·8 to 37·2 μg. per 24 hours for oestrogen excretion and from 0·9 to 2·1 mg. per 24 hours for pregnanediol excretion. The average (±SD) maximum, mean and standard deviations of urinary oestrogen and pregnanediol excretion for the 20 ovulatory cycles are included in Table II.

### *Anovulatory patients studied by daily collections*

The values for oestrogen and pregnanediol excretion obtained daily throughout a complete anovulatory cycle are shown in Figure 1. The mean oestrogen and pregnanediol values for the complete cycle calculated from the daily values and from the weekly combinations are shown in Table I. No significant difference was demonstrated between any of these means.

The corresponding values obtained over a random interval of 28 days in a patient with secondary amenorrhoea are shown in Figure 2 and Table I. No significant differences were demonstrated between the mean oestrogen and pregnanediol values calculated from daily monitoring and weekly tracking. However, in the weekly tracking, some differences were encountered in the mean oestrogen values which

TABLE II

*Average maximum, mean and standard deviations of urinary oestrogen and pregnanediol excretion in the anovulatory patients listed according to the clinical disorder compared with the values found in ovulatory cycles*

| Clinical disorder | No. of patients | Urinary oestrogen excretion (μg./24 hours) | | | Urinary pregnanediol excretion (mg./24 hours) | | |
|---|---|---|---|---|---|---|---|
| | | maximum | mean | standard deviation | maximum | mean | standard deviation |
| Primary amenorrhoea | 9 | 9·3±10·4 | 6·6±7·8 | 2·0±2·0 | 0·49±0·35 | 0·36±0·31 | 0 11±0·07 |
| Secondary amenorrhoea | 117 | 14 9±12·4 | 10·2±8·6 | 3 5±3·2 | 0·60±0·30 | 0·40±0·18 | 0·14±0·11 |
| Oligomenorrhoea | 99 | 34·8±21·5 | 21·3±9·9 | 9·5±8·2 | 0·85±0·49 | 0·49±0·22 | 0·23±0·19 |
| Anovulatory cycles | 33 | 35·1±21·4 | 18·6±9·3 | 11·2±8·0 | 0·98±0·46 | 0·60±0·28 | 0·29±0·21 |
| Ovulatory cycles | 20 | 52·5±15·3 | 25·7±7·2 | 13·3±3·9 | 4·70±1·30 | 1·50±0·50 | 1·40±0·40 |

All values are means ± standard deviation for each group.

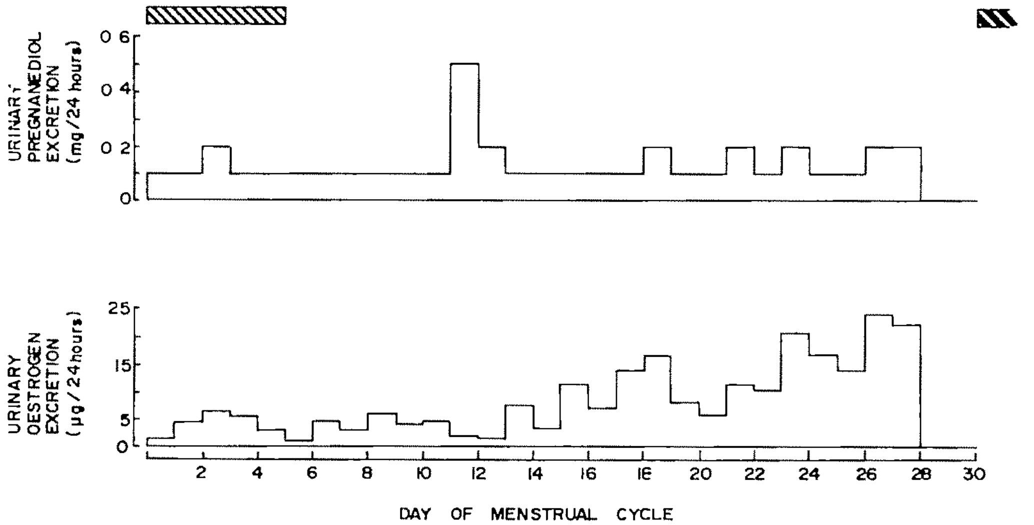

FIG. 1

Urinary oestrogen and pregnanediol excretion during an anovulatory menstrual cycle. The cross-hatched horizontal bars indicate menstruation.

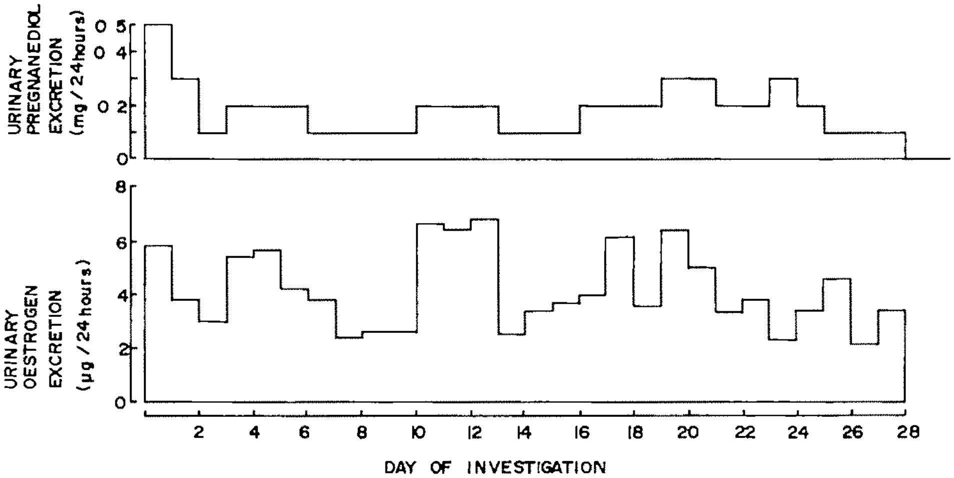

FIG. 2

Urinary oestrogen and pregnanediol excretion during four weeks in a subject with secondary amenorrhoea of 12 months' duration. Menstruation did not occur within the 3 months following this study.

depended on the day on which tracking started; thus the mean value was different when calculated from tracking starting on day 2 compared with day 4 ($0{\cdot}05 > p > 0{\cdot}025$), and also on day 3 compared with day 4 ($0{\cdot}025 > p > 0{\cdot}02$) and on day 3 compared with day 5 ($0{\cdot}05 > p > 0{\cdot}025$).

*Patients studied by weekly tracking*

The numbers of patients who ovulated during the tracking period as judged by the pregnanediol values are shown in Table III. Fifteen of the 132 patients (11·4 per cent) with secondary amenorrhoea ovulated during tracking; five of these were shown to have Asherman's syndrome and the other 10 menstruated subsequently at the time expected from the hormone analyses; only one of these 10 patients had had amenorrhoea for longer than two years. Thirty-nine of the 138 patients (28·3 per cent) with oligomenorrhoea ovulated during tracking; 18 of the patients who ovulated had cycle lengths of 6 to 8 weeks, 15 had cycle lengths of 9 to 12 weeks and 6 had cycle lengths of 3 to 6 months. Of the 227 patients with cycle lengths of 25 to 42 days, 194 (85·4 per cent) ovulated during the period of tracking and the other 33 had anovulatory cycles.

TABLE III

*Categorization of the 506 patients studied and the incidence of ovulation during the tracking prodecure*

| Clinical disorder | Number of patients | | |
|---|---|---|---|
| | Anovulatory | Ovulatory | Total |
| Primary amenorrhoea | 9 | – | 9 |
| Secondary amenorrhoea | 117 | 15 (11%) | 132 |
| Oligomenorrhoea | 99 | 39 (28%) | 138 |
| Cycle 25 to 42 days | 33 | 194 (85%) | 227 |
| Total | 258 | 248 (49%) | 506 |

The patients who did not ovulate were classified according to whether they had primary or secondary amenorrhoea, oligomenorrhoea or anovulatory cycles. The maximum and mean oestrogen and pregnanediol values and the standard deviations of the means were tabulated for each patient and the means and standard deviations for these figures were calculated for each of the four clinical disorders. The results are included in Table II. Significant negative correlations ($p < 0{\cdot}001$) were found between the maximum, mean and standard deviation of the oestrogen and pregnanediol values and the severity of the anovulatory symptom. The major portion of this correlation was contributed by the differences between the groups with secondary amenorrhoea and those with oligomenorrhoea. Although the trend was evident between the patients with primary and secondary amenorrhoea, the differences did not reach statistical significance. The three assessments of oestrogen and pregnanediol excretion in the patients with oligomenorrhoea were not significantly different from those found in the patients with anovulatory cycles (Table II).

The mean urinary oestrogen values in the 20 normal ovulating women were significantly higher than those found in the patients with primary amenorrhoea ($p<0\cdot001$), secondary amenorrhoea ($p<0\cdot001$) and anovulatory cycles ($0\cdot01>p>0\cdot005$) but were not significantly different from those found in the patients with oligomenorrhoea. The mean pregnanediol values were significantly lower in all of the anovulatory groups compared with the women who ovulated ($p<0\cdot001$) (Table II).

The maximum and mean urinary oestrogen values were positively correlated with the presence of hirsuties ($0\cdot01>p>0\cdot001$) and with excessive weight ($p<0\cdot001$). Most of this correlation was contributed by the patients with secondary amenorrhoea ($0\cdot05>p>0\cdot01$); the trend was still evident in patients with oligomenorrhoea and anovulatory cycles but the differences were not significant. The effect of loss of weight on the oestrogen values of obese patients was not evaluated.

The maximum and mean pregnanediol values correlated with each other and with the maximum and mean oestrogen values (all $p<0\cdot001$). Maximum pregnanediol values also correlated with the occurrence of menstruation during or within two months following tracking ($p<0\cdot001$) the major portion of this correlation was contributed by the patients with oligomenorrhoea and anovulatory cycles.

No significant correlations were found between the urinary oestrogen and pregnanediol values and the patients' ages or the presence or absence of galactorrhoea. The finding of Shearman and Turtle (1970) that basal urinary oestrogen values were higher in patients with secondary amenorrhoea and galactorrhoea when the conditions followed the use of oral contraceptives was not confirmed.

*Patients with primary amenorrhoea*

The maximum and mean urinary oestrogen and pregnanediol values found in the nine patients with primary amenorrhoea are shown in Figure 3 according to whether menstruation subsequently occurred during a follow-up period of two years or more. In the seven patients in whom the amenorrhoea persisted, the maximum oestrogen values were all less than 8 $\mu$g. per 24 hours and the mean values were less than 5 $\mu$g. per 24 hours; the corresponding pregnanediol values were 0·6 mg. per 24 hours and 0·35 mg. per 24 hours. These values can be taken as representing absence of ovarian activity, and a single urine specimen would have provided a representative assessment. The clinical details of the other two patients with higher values merit documentation. One, aged 27 years, had maximum mean and minimum oestrogen values of 29 $\mu$g., 21 $\mu$g. and 10 $\mu$g. per 24 hours respectively and spontaneous menstruation occurred within one month after tracking. The other, aged 19 years, had maximum, mean and minimum oestrogen values of 28 $\mu$g., 21 $\mu$g. and 16 $\mu$g. per 24 hours and spontaneous menstruation occurred within two years after tracking. These two patients have not been excluded from the calculations.

No overlap was found between the oestrogen values in the two patients who subsequently menstruated spontaneously and the others who remained amenorrhoeic. Thus individual urinary oestrogen values greater than 10 $\mu$g. per 24 hours in this group were suggestive that spontaneous menstruation would occur within two years.

*Patients with secondary amenorrhoea*

The maximum pregnanediol value recorded in the 117 patients with secondary amenorrhoea who were anovulatory during tracking was 1·6 mg. per 24 hours and only 14 patients recorded maximum values of 1·0 mg. per 24 hours or more. The oestrogen values obtained in the two patients with the highest pregnanediol values showed clearly that ovulation could not have occurred during the tracking interval.

The mean oestrogen value and the standard deviation from the mean for each of the 117 patients who did not ovulate during the tracking interval are plotted according to the duration of amenorrhoea in Figures 4 and 5. The mean oestrogen values were negatively correlated with the duration of amenorrhoea ($p<0\cdot001$). A similar correlation was found for the maximum

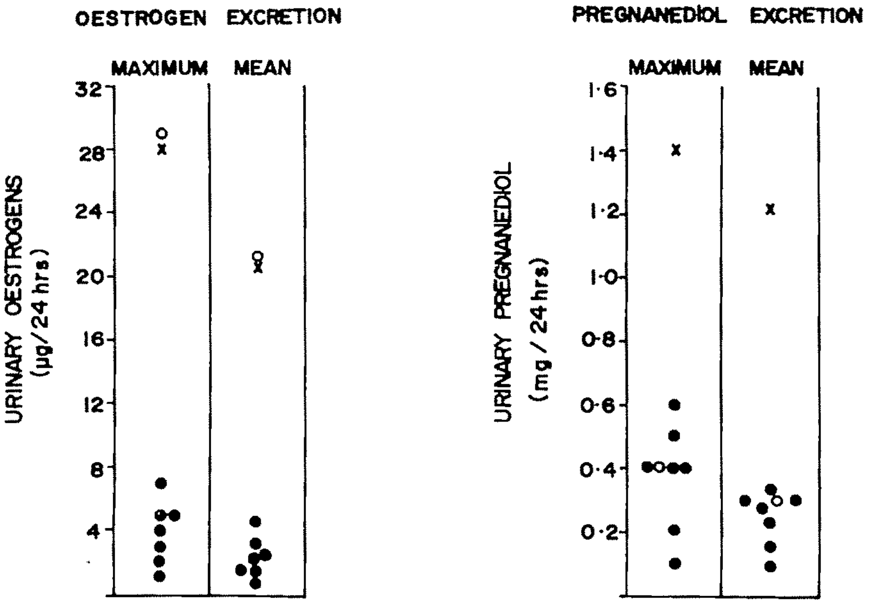

FIG. 3

Maximum and mean oestrogen and pregnanediol excretion in nine patients with primary amenorrhoea. The values marked with an "X" and an "O" are those in the two subjects who subsequently menstruated spontaneously.

oestrogen value and the duration of amenorrhoea ($p<0{\cdot}001$). The patients subsequently shown to have ovarian failure as indicated by high gonadotrophin values (marked "X") were excluded from the calculations.

The variability between the oestrogen values found in an individual also declined as the duration of amenorrhoea increased but the differences were not significant. No differences were found in the oestrogen or pregnanediol values of the patients whose amenorrhoea followed the use of oral contraceptives compared with those in whom oral contraception was not a factor.

To evaluate the usefulness of a single urine specimen as an indication of the mean oestrogen excretion during tracking in patients with secondary amenorrhoea, each oestrogen value obtained in a patient was compared with the mean value for that patient. The results were as follows: (1) 71 patients had one or more oestrogen values less than 5 µg. per 24 hours; of these only five had mean values greater than 10 µg. per 24 hours and all of these five patients had had amenorrhoea for less than two years; (2) 67 patients had one or more oestrogen values between 6 µg. and 10 µg. per 24 hours; 16 of these had mean values greater than 10 µg. per 24 hours and all except one of these 16 patients had had amenorrhoea for less than two years; (3) 49 patients had one or more oestrogen values between 11 µg. and 15 µg. per 24 hours; 29 of these had mean values greater than 10 µg. per 24 hours; this division was not correlated with the duration of amenorrhoea; (4) 55 patients had one or more oestrogen values greater than 15 µg. per 24 hours; of these only two patients had mean values of 10 µg. per 24 hours or less and both had had amenorrhoea for less than two years.

Thus, in the patients with secondary amenorrhoea, one urine specimen with an oestrogen value of 10 µg. per 24 hours or less provided a useful assessment of the mean oestrogen excretion in 93 per cent of patients and in 100 per cent of patients when the duration of amenorrhoea exceeded two years. However, more than one specimen was required to obtain an accurate assessment of the overall oestrogen values when the single value exceeded 10 µg. per 24 hours. In

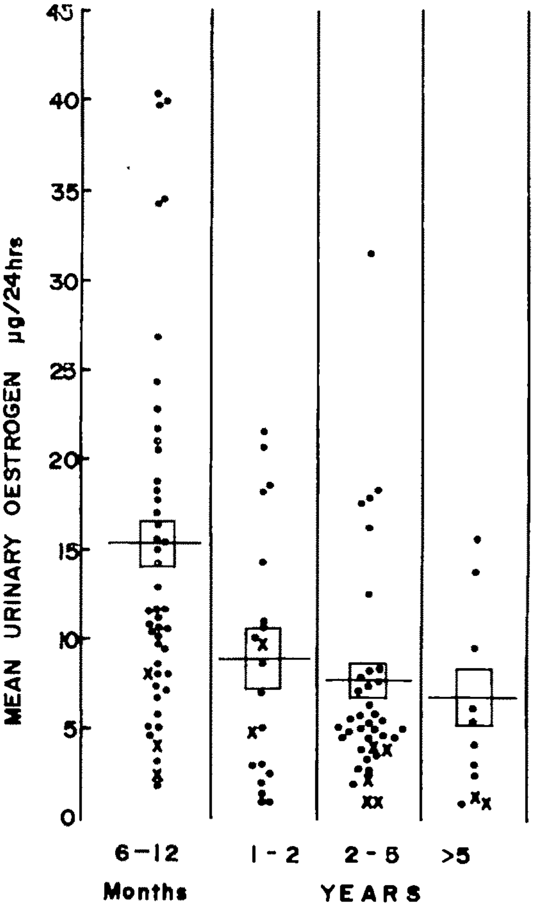

FIG. 4

Mean urinary oestrogen excretion in relation to duration of secondary amenorrhoea. The values marked with an "X" represent those of patients subsequently proven to have ovarian failure. The horizontal lines represent the mean values and the boxes represent the standard error of the mean of the group.

the case of pregnanediol estimations, a single urine specimen provided a useful estimate of the mean pregnanediol value when the oestrogen value in that specimen was 10 µg. per 24 hours or less.

The mean oestrogen value and the standard deviation of the mean for each patient are plotted in Figure 6 according to whether the amenorrhoea persisted (87 patients) or whether menstruation occurred during or within two months after tracking (4 patients). Analysis of variance showed significant differences between the presence or absence of menstruation and the mean oestrogen values and also the variability of the oestrogen values. A discriminant function ($f$) was determined mathematically to separate the patients who bled from those who did not. The formula obtained was:

$$f = \text{standard deviation} - 0{\cdot}12 \times \text{mean value}.$$

The line of maximum demarcation is given when $f = 13{\cdot}37$ and is shown in Figure 6. Patients with mean and standard deviation values above this line were more likely to menstruate during or within two months after tracking than those with values below the line.

*Patients with oligomenorrhoea*

Of the 99 patients with oligomenorrhoea who were classified as being anovulatory during

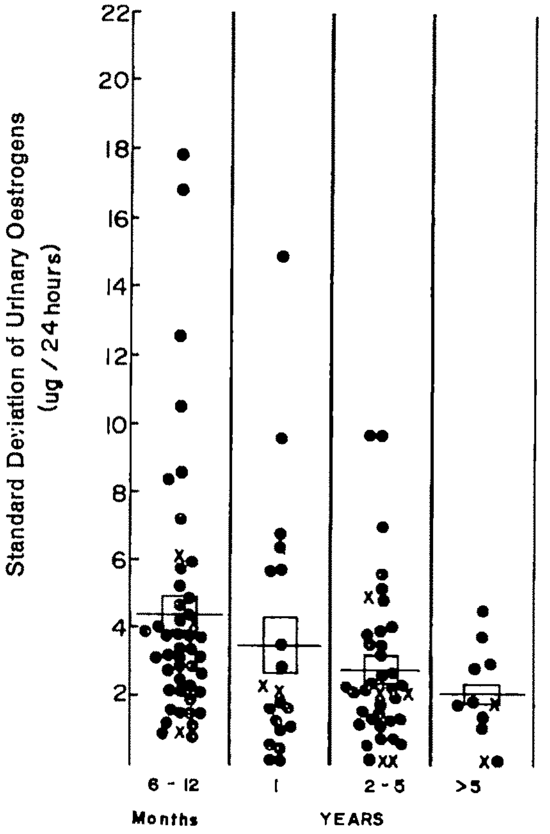

FIG. 5

Variability (standard deviation) in oestrogen excretion in relation to the duration of the secondary amenorrhoea. The values marked with an "X", the horizontal lines and the boxes represent the same features as in Figure 4.

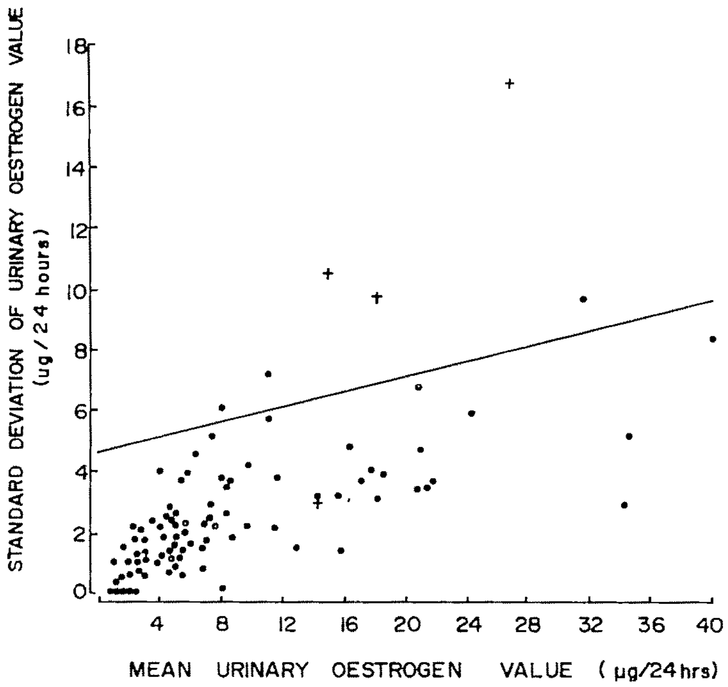

FIG. 6

Menstrual data in patients with secondary amenorrhoea correlated with the mean oestrogen value and the variability (standard deviation) of the oestrogen values. The values marked + belong to patients who had menses within two months. The values marked ● denote patients who had no menses within two months of tracking. The line of maximum demarcation is shown.

weekly tracking, 10 had maximum pregnanediol values of 1·8 mg. to 1·9 mg. per 24 hours. Two of these 10 patients recorded double oestrogen peaks and it is possible that they ovulated; however, they were not excluded from the following analysis of results.

The mean oestrogen values in the 99 anovulatory patients with oligomenorrhoea are listed in Figure 7 according to the patient's usual cycle length. Only 9 patients had mean oestrogen values of 10 μg. per 24 hours or less; none of these menstruated during or within 2 months after tracking and subsequently four were shown to have developed ovarian failure. The mean oestrogen values did not correlate with cycle length and were not significantly different from those found in normal subjects with ovulatory cycles. However, significant negative correlations were obtained between cycle length and the maximum oestrogen values and also between cycle length and the variability in the oestrogen values (both $0{\cdot}01 > p > 0{\cdot}001$). The major portion of these correlations was contributed by the comparison between the cycles of less than three months' duration with those of more than three months' duration.

The mean oestrogen values and the standard deviations of the values for 71 of these patients who did not ovulate are correlated in Figure 8 with the occurrence of bleeding during or within two months after tracking. A discriminant function (f) calculated from the data, was represented by the formula:

f = standard deviation $-0{\cdot}21 \times$ mean value. Of the 62 patients with mean oestrogen values greater than 10 μg. per 24 hours, 23 had mean values and standard deviations above the line of maximum demarcation and 20 of these bled, whereas 39 had values below that line and 18 of these bled. This difference was significant

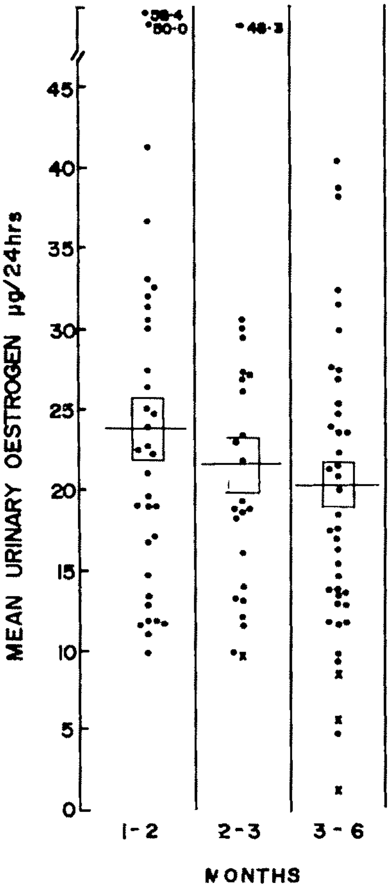

FIG. 7
Mean urinary oestrogen excretion in relation to usual cycle length in patients with oligomenorrhoea. The values marked with an "X", the horizontal lines and the boxes represent the same features as in Figure 4.

(p<0·01). Therefore, the higher the oestrogen value and the greater its fluctuation, the more likely the patient was to bleed. In this group a single urine specimen was of no value in assessing the mean oestrogen excretion.

*Patients with anovulatory cycles*

Of the 33 patients who were classified as having anovulatory cycles of 24 to 42 days duration, two had maximum pregnanediol values of 1·8 mg. per 24 hours and may have ovulated.

The maximum and mean oestrogen values obtained during weekly tracking (Table II) were significantly lower than the values observed in ovulatory cycles (0·005>p>0·001 respectively). The variability in oestrogen values was not significantly different from that seen during ovulatory cycles or in patients with oligomenorrhoea and a single urine specimen was of no value in assessing the mean oestrogen excretion.

## DISCUSSION

The basic patterns of urinary oestrogen and pregnanediol excretion in the various classifications of ovarian function have already been documented (Brown and Matthew, 1962). These have ranged from low more or less constant values characteristic of absent ovarian function to the higher oestrogen values which may be constant or fluctuating found in anovulatory menstrual cycles and to the fluctuating oestrogen and pregnanediol values found in ovulatory cycles. The present study demonstrated that other aberrant patterns which appear to be associated with infertility may sometimes be encountered. However, the main emphasis of the study has been to provide mathematical confirmation of the above earlier findings.

For the analytical method employed here, a pregnanediol value which rose to 2·0 mg. per 24 hours or more and was maintained at these levels for at least 7 days was accepted as indicating that ovulation had occurred. This dividing point was not absolute and it is possible that several women with pregnanediol values within the range of 1·5 to 1·9 mg. per 24 hours also ovulated. On this criterion, approximately 50 per cent of the 529 women studied, ovulated. The majority of these were women with menstrual cycle lengths of 24 to 42 days, in whom the incidence of ovulation was 87 per cent. This indicates that cycles of 24 to 42 days in length are usually ovulatory, even though prior evidence of an abnormality has been obtained.

For the oestrogen method used here, values of 10 μg. per 24 hours or less were associated with continuing amenorrhoea of two months or more, indicating that the ovaries were inactive at the time, and that this inactivity was likely to persist. This was illustrated by the 9 patients with primary amenorrhoea; all 7 patients with continuing amenorrhoea had oestrogen values of less than 10 μg. per 24 hours and the two who later menstruated spontaneously had fluctuating oestrogen values which exceeded 20 μg. per 24 hours. The mean and maximum oestrogen

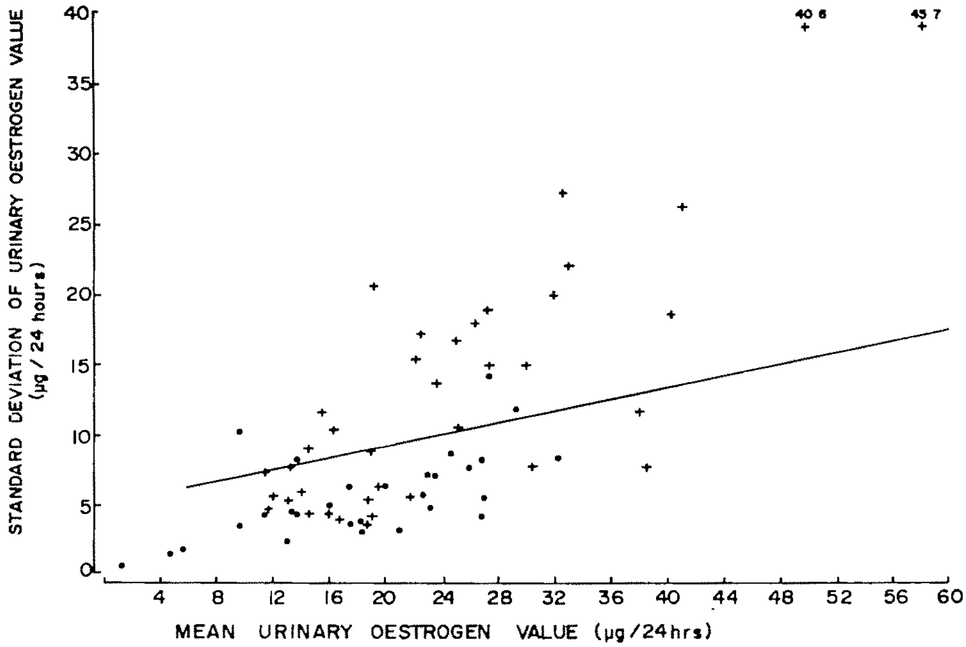

Fig. 8

Menstrual data in patients with oligomenorrhoea correlated with the mean oestrogen value and the variability (standard deviation) of the oestrogen values. The values marked + belong to patients who had menses within two months of tracking. Those marked ● belong to patients with no menses within two months of tracking. The line of maximum demarcation is included.

values and the fluctuation in values found in the various clinical classifications correlated directly with increasing ovarian activity as indicated by the menstrual findings. Thus the mean oestrogen value in the patients with primary amenorrhoea was 6·6 µg. per 24 hours, in the patients with secondary amenorrhoea it was 10·2 µg. per 24 hours and in the patients with oligomenorrhoea, anovulatory cycles and normal cycles it was within the range 18·6 µg. to 25·7 µg. per 24 hours (Table II). None of the patients with secondary amenorrhoea and mean oestrogen values of 10 µg. per 24 hours or less in the 4 to 6 weekly samples bled during the 2 month follow-up period, and 54 of the 69 patients (75 per cent) with amenorrhoea of more than a year's duration had mean oestrogen values of 10 µg. per 24 hours or less. This dividing point also applied to the patients with oligomenorrhoea; none of the 9 patients with oestrogen values of 10 µg. per 24 hours or less menstruated within two months of the investigation and 4 of these were subsequently shown to have ovarian failure.

For oestrogen values above 10 µg. per 24 hours the picture was less clear and it was necessary to include the variability of the oestrogen values in the calculation. A line of maximum demarcation calculated on this basis separated the patients into those who were likely to bleed (high values) from those who were less likely to bleed (low values). For example, in the patients with oligomenorrhoea, 87 per cent with values above this line bled, whereas only 46 per cent with values below the line bled. Failure to bleed at higher values could have been due to the phenomenon that bleeding does not necessarily follow anovulatory ovarian activity although it almost always follows ovulatory activity providing a responsive endometrium is present.

The data presented here show that four to six oestrogen and pregnanediol values obtained at weekly intervals provide a considerable amount of information on ovarian activity occurring during the period of study. Although daily monitoring provided the additional information on the probable day of ovulation, weekly tracking provided a reliable estimate of the mean daily values and of their variability in all the categories of patients studied, and also showed whether ovulation was occurring or not. Beavis *et al.* (1970) successfully used the procedure to investigate ovarian function in premenopausal women after hysterectomy with conservation of the ovaries. For economy of effort, weekly collections could be discontinued in those patients who ovulated, as soon as the luteal phase rise in pregnanediol excretion had been identified.

There were two circumstances in which a single oestrogen and pregnanediol determination gave the same information as that provided by weekly tracking. In the patients with regular menstruation, the occurrence of ovulation was indicated by the finding of a pregnanediol value of 2·0 mg. per 24 hours or more in a single urine specimen collected between 4 and 8 days inclusive before the onset of bleeding. This group represented 45 per cent of the patients studied. This information was therefore similar to that provided by an endometrial biopsy performed premenstrually. In the absence of a demonstrated pregnanediol rise, it was of course necessary to check that menstruation did in fact occur on the expected date in case the collection had been mis-timed. The other instance in which a single specimen gave an accurate indication of the overall picture was in the patient with amenorrhoea of greater than two years duration in whom the oestrogen value was 10 μg. per 24 hours or less. Such a finding was invariably associated with a low pregnanediol value and indicated that the ovarian inactivity was likely to persist unless treatment was instituted. In all other circumstances multiple sampling was necessary to obtain a valid picture of ovarian activity. Nevertheless, with the appreciation of these factors it is possible to obtain the maximum information with the greatest economy of effort when studying ovarian function in patients with disorders of menstruation and associated infertility.

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# FUNCTIONAL AND POST-ORAL CONTRACEPTIVE AMENORRHOEA: RESPONSE TO LUTEINIZING HORMONE RELEASING HORMONE (LH-RH)

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## Summary

Basal levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH) in serum and their response to the luteinizing hormone releasing hormone (LH-RH) were measured in a group of patients with functional amenorrhoea. The results in this group were compared to those obtained in a second group of patients with secondary amenorrhoea which had followed cessation of a combined oral contraceptive. No difference either in the basal levels or in the response to LH-RH could be found. These data indicate that there is no detectable difference in pituitary gonadotrophin status between the two groups and that the two types of amenorrhoea have the same aetiological background.

IT has been shown that there is a dose-related release of LH and FSH from the pituitary in response to luteinizing hormone releasing hormone (LH-RH) administration (Kastin *et al.*, 1971; Besser *et al.*, 1972). More recently it has been suggested that LH-RH may be responsible for the synthesis (Mittler *et al.*, 1970; Redding *et al.*, 1972; Mortimer *et al.*, 1974) as well as the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH). Since the pituitary response to LH-RH must, in part, depend on the pituitary content of LH and FSH, the gonadotrophin levels observed during the test must reflect the levels of endogenous secretion of the releasing hormone. Indeed, we have previously reported impaired responses of LH and FSH in patients with destructive lesions in the hypothalmic region (Jequier *et al.*, 1974) and this test therefore has considerable value as an investigative tool for the assessment of patients with reproductive disorders.

The purpose of this study was to compare the basal levels of LH and FSH and their response to exogenous LH-RH in the two groups of patients with secondary amenorrhoea in an attempt to elucidate the aetiology of the anovulation.

## PATIENTS

Two groups of patients were studied: one consisted of 12 patients with functional amenorrhoea; the other was made up of six patients with secondary amenorrhoea that dated from cessation of treatment with combined oral contraceptive. No patient in the post-oral contraceptive group gave a previous history of amenorrhoea and in every case the amenorrhoea had lasted for more than six months. None of the patients with functional amenorrhoea had taken the oral contraceptive within two years of the onset of the amenorrhoea. The patients whose secondary amenorrhoea dated from cessation of combined oral contraceptive therapy had all been taking a combination of 50 μg. of ethinyl oestradiol and 2·5 mg. of a progestogen.

A general as well as a gynaecological examination had been made and investigations included an X-ray of the pituitary fossa, thyroid function tests, measurement of 24-hour urinary excretion of 17 oxogenic and oxosteroids, serum gonadotrophins and plasma oestradiol concentrations. Patients in whom there was evidence of ovarian failure or in whom an anatomical lesion in the hypothalmic-pituitary region was found were excluded from this study.

### *LH-RH test*

The LH-RH used was a synthetic preparation. A dose of 100 μg. was given as an intravenous bolus and blood was obtained from an antecubital vein at zero time and at 20, 40 and 60 minutes thereafter. The blood was allowed to clot at 4 °C., separated and the serum stored at −20 °C.

### *Assay of LH and FSH*

Serum LH and FSH were measured by a double antibody radioimmunoassay (Jacobs and Lawton, 1974). LH was measured using an anti-HCG serum supplied by Dr. W. D. Odell and the results were expressed in terms of the MRC 68/40 preparation. FSH was measured using an HCG-adsorbed anti-FSH serum supplied by Dr. W. D. Odell and the results expressed in terms of the MRC 68/39 preparation. In the tubes containing the standards an equal volume of serum from hypophysectomized or oestrogen-treated men replaced the unknown serum

## RESULTS

The individual responses of the patients in the two groups are shown in Figure 1, which also shows the mean of the peak levels (± one Standard Error of this mean). The range of responses of LH and FSH to LH-RH were wide in both groups of patients.

No difference could be found in either the basal levels of LH or FSH or in their responses to the gonadotrophin releasing hormone.

## DISCUSSION

An association between combined oral contraceptive therapy and amenorrhoea following its use was first suggested by Shearman (1966). Since then other authors (Rice-Wray *et al.*, 1967; Friedman and Goldfien, 1969; Larsson-Cohn, 1969; Halbert, 1971; Gambrell *et al.*, 1971; Golditch, 1972; Shearman and Smith, 1972; Steele *et al.*, 1973) have found a high incidence of amenorrhoea associated with cessation of combined oral contraceptives. Most of these authors were working in specialist units and were therefore studying selected patients. In fact, Venning (1973) quotes studies by Treloar that show no statistical difference in the incidence of amenorrhoea in patients who have and those who have never taken an oral contraceptive. The fact that no specific constituent of the oral contraceptive has ever been identified as a causative agent, and that post-oral amenorrhoea appears to be unrelated to the duration of therapy (Halbert, 1971) casts doubt on any causal relationship that amenorrhoea may have with the oral contraceptive.

Treloar *et al.* (1967) in their very large study of menstrual intervals, demonstrated a high incidence of menstrual irregularities for some six to eight years after the menarche, and, since this is a very common time for the use of oral contraceptives, their observation may account for some of the association between amenorrhoea and the contraceptive pill. Increased cycle lengths are often recurrent (Treloar *et al.*, 1967) which may explain the higher incidence of post-oral contraceptive amenorrhoea which has been reported in patients with a past history of menstrual irregularities.

In the two groups of patients reported here, no difference in either the basal levels or in the responses to LH-RH was found. Although the range of individual responses was wide, the means of the peak values of both LH and FSH were indistinguishable in the two groups. In other types of secondary anovulation, such as occur in hypothalmic disease, hypopituitarism, anorexia nervosa and ovarian failure, the LH-RH responses show different patterns that can, by comparing the mean peak values of LH and FSH, be distinguished from those seen in functional amenorrhoea and amenorrhoea following the use of the oral contraceptive (Jequier *et al.*, 1974). The similarity in basal levels of LH and FSH, as well as in the responses to LH-RH support the authors' belief that functional amenorrhoea and amenorrhoea following cessation of oral contraceptive therapy cannot be

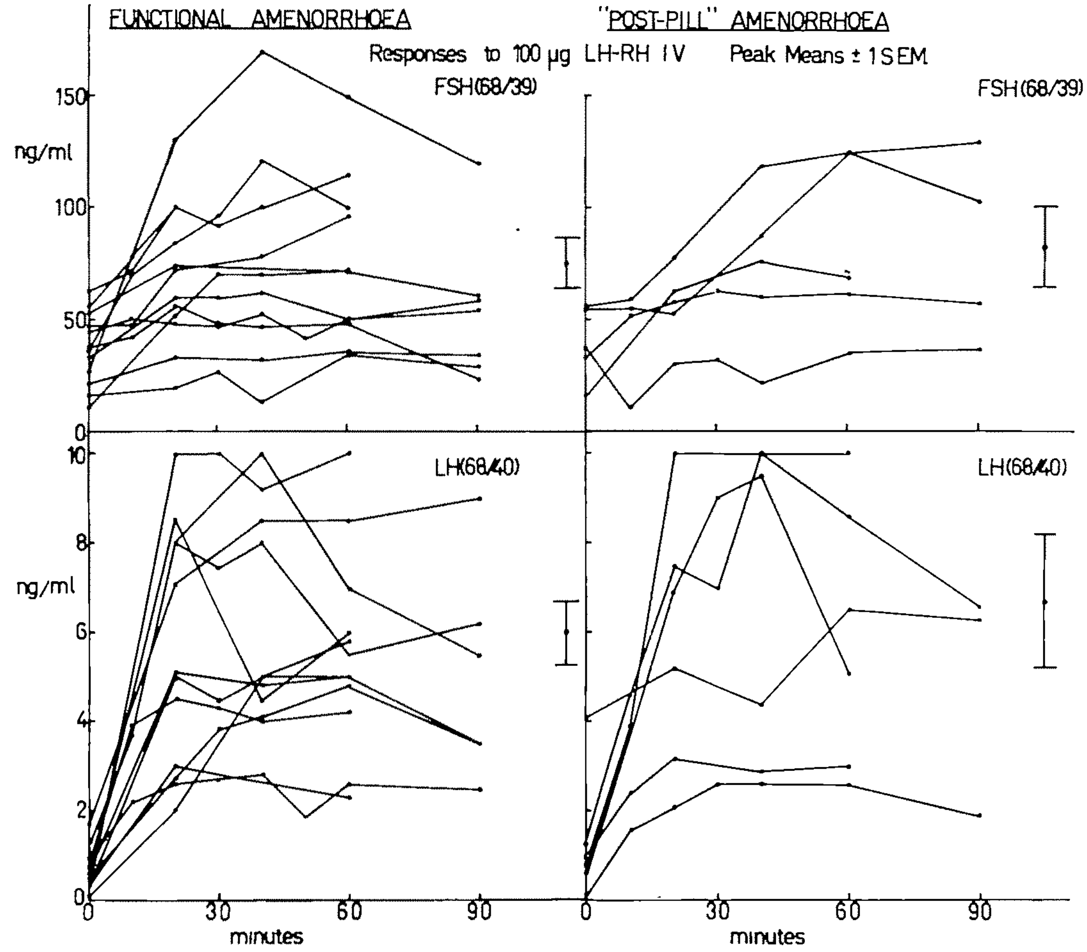

Fig. 1
FSH and LH levels after LH-RH in the two groups of patients.

distinguished. Furthermore, we believe that a causal relationship between anovulation and cessation of the combined oral contraceptive has yet to be proved.

## ACKNOWLEDGEMENTS

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# PATHOLOGICAL MECHANISMS TO EXPLAIN SOME CASES OF AMENORRHOEA WITHOUT ORGANIC DISEASE

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## Summary

A test is described to define the site of the lesion causing amenorrhoea in women of child-bearing age who have normal ovaries and a normal response to luteinizing hormone-releasing hormone (LH-RH). Patients able to produce a release of LH following the administration of 1 mg. of oestradiol benzoate (EB) have normal hypothalamic function and thus the site of the defect must lie more centrally in the brain. Patients unable to show these LH surges have either a primary or secondary abnormality affecting their hypothalamic oestrogen "receptor" mechanisms or their ability to produce LH-RH.

THE availability of a polypeptide with gonadotrophin releasing activity (luteinizing hormone-releasing hormone or LH-RH) has made it possible to test the functional ability of the pituitary gland to produce luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Nillius and Wide, 1972; Bonnar and MacKinnon, 1972; Nakano *et al.*, 1973; Zarate *et al.*, 1973; Mortimer *et al.*, 1973). In a woman with normal ovaries, in the presence of amenorrhoea and a normal pituitary response to LH-RH, the abnormality must then lie at a higher level than the pituitary gland.

Oestradiol has been shown to release gonadotrophins in normal women (Tsai and Yen, 1971; Yen and Tsai, 1972; Monroe *et al.*, 1972) and in some women with amenorrhoea (Nillius and Wide, 1971; Shaw *et al.*, 1975). This action of oestradiol is probably mediated via the hypothalamus and evidence to support this contention comes from animal work in which oestrogen implants were placed in the hypothalamus (Palka *et al.*, 1966; Motta *et al.*, 1968).

We have therefore used an oestrogen provocation test to exclude oestrogen feedback hypothalamic dysfunction as the cause of amenorrhoea in a way analogous to the use of LH-RH to exclude gonadotrophin deficiency within the pituitary as a basis for the problem.

## METHODS

Twenty-three subjects with secondary amenorrhoea of between 12 and 98 months' duration were investigated. Disease of the pituitary, ovaries, thyroid and adrenals was excluded by a normal skull X-ray and normal serum estimations of protein bound iodine (PBI), thyroid stimulating hormone (TSH), diurnal cortisols, testosterone, $\Delta^4$ androstenedione, sex hormone binding globulin, LH, FSH, prolactin, oestradiol and progesterone. All the subjects had FSH and LH responses to LH-RH that were either within or above the levels found in normal women studied between days 4 and 6 of their menstrual cycles (Shaw *et al.*, 1974). A laparoscopy and ovarian biopsy were performed to exclude any primary ovarian pathology. The subjects were divided into three clinically distinct groups.

*Anorexia Nervosa* (*7 subjects*). All had received in-patient treatment for this condition and amenorrhoea had begun during the period of acute weight loss. Although none were in the acute phase of the illness at the time of the tests and had regained some weight, all were still below 85 per cent of their ideal body weight for height.

*Post-pill amenorrhoea* (*8 subjects*). The amenorrhoea had begun after stopping combined oral contraceptive agents. The duration of oral contraceptive therapy had ranged between 6 and 36 months. Five of the subjects had had regular menses prior to their taking the pill whilst the other three had had irregular cycles.

*Psychogenic amenorrhoea* (*8 subjects*). In these women amenorrhoea had commenced at a time of intense emotional upheaval such as marriage break-up, final examinations, or death of a close relative. There was no history of oral contraception or weight loss.

### *Test procedures*

*LH-RH test.* The response of the pituitary gland to 100 μg. of LH-RH was evaluated by estimating serum LH and FSH 15 and 0 minutes before and 20, 30 and 60 minutes after the intravenous injection of the LH-RH. Basal serum oestradiol-17β ($E_2$) and progesterone (P) were measured. This test was usually performed on an out-patient basis.

*Oestrogen provocation test.* A 1 mg. dose of oestradiol benzoate (EB) was given intramuscularly and serum samples for LH and FSH measurements taken 0, 8, 24, 32, 48, 56, 72, 82 and 96 hours later. $E_2$ and P were also estimated on the first sample. A positive response was defined as an increase of more than 8 u/l. above baseline values for LH and of 3 u/l. for FSH (LH and FSH were measured in u/l. against the standard reference preparation MRC 69/104). The circulating levels of $E_2$ achieved at various time intervals after the EB injection have also been published elsewhere (Shaw *et al.*, 1975). The specificity of the assays, antisera and materials used for labelling have previ described (Shaw *et al.*, 1974).

## RESULTS

The basal $E_2$ and P values in tl groups are shown in Table I. No difference existed in these basal level any of the groups studied here or betv groups and the values seen in 30 norm tested during the early follicular pha menstrual cycle (day 4 to 6). This normal women were also used to normal ranges of response to 100 μg. for comparison with the amenorrhoei

TABLE I

*Basal levels of oestradiol-17β ($E_2$) and prog at the time of the LH-RH tests in the group women acting as controls (Day 4 to 6 of men and the various groups of subjects with ame*

| Clinical groups | No., | Basal le<br>$E_2$ (pmol./l.) | ( |
|---|---|---|---|
| | | Mean ± SD | I |
| Controls | 30 | 203 ± 140 | |
| Anorexia nervosa | 7 | 189 ± 77 | |
| Post-pill amenorrhoea | 8 | 226 ± 96 | |
| Psychogenic amenorrhoea | 8 | 315 ± 210 | |

The LH responses to the LH-RH a gen provocation tests are shown in Fi can be seen from the LH-RH test resp upper limit of the normal range was in 10 of the 23 subjects studied. Tho with exaggerated responses to LH-RH identifiable clinically.

A positive LH surge in response to t gen provocation test was seen in 6 pa began 48 hours after the EB injecti remained elevated until 72 hours. Th were seen in 2 of the 8 with post-pill am and 4 of the 8 with psychogenic am (Fig. 1). The size of the LH-RH respon appear to govern the manner in whic would respond to the oestrogen pr test, nor were the basal $E_2$ values rela responses.

FSH responses to the various stimul

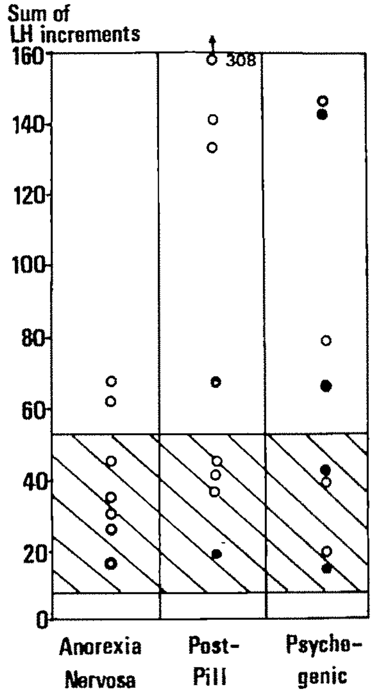

FIG. 1
Responses to 100 μg. of LH-RH, expressed as sum of LH increments (20, 30 and 60 minutes), in the various subjects with amenorrhoea. Cross-hatched area shows range of response in control group (see text). ● indicates patients who also demonstrated LH release after oestrogen provocation test.

are shown in Figure 2. Six subjects demonstrated exaggerated responses to LH-RH, three with post-pill and three with psychogenic amenorrhoea. Only four subjects, all of whom were suffering from psychogenic amenorrhoea, showed positive FSH responses to the oestrogen provocation test. All of these also had LH responses to oestradiol but the peak FSH levels did not always coincide with the peak LH levels.

## DISCUSSION

Oestrogens have two differing effects upon gonadotrophin release in normal women. There is initially a suppression of LH levels followed by a subsequent elevation above baseline values (Tsai and Yen, 1971; Yen and Tsai, 1972; Monroe *et al.*, 1972; Shaw, 1975). These changes are thought to be mediated by LH-RH release from the hypothalamus. Thus in females with normal pituitary function, the oestrogen provocation test with LH measurement could theoretically be used as a test of hypothalamic function. In patients suffering from amenorrhoea, but with a normal LH surge following oestrogen provocation, the site of any defect must lie outside the hypothalamic-pituitary axis. On the other hand, those unable to produce LH release will have a defect either in the hypothalamus or hypothalamic-pituitary connections. Another possibility is that the lesion may be at a higher level than the hypothalamus for there is evidence that higher centres within the brain tonically sustain hypothalamic function, thus disease within these areas may lead to secondary dysfunction within the hypothalamus (Taleisnik, 1966; Sawyer, 1966).

In the groups of subjects here investigated, all seven with anorexia nervosa failed to respond to

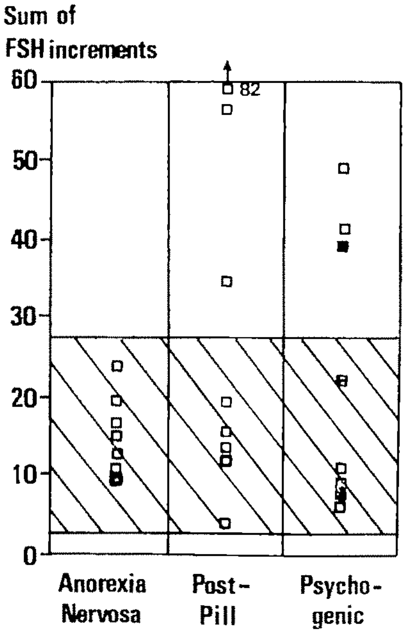

FIG. 2
FSH response to LH-RH test, expressed as sum of increments at 20, 30 and 60 minutes, in the patients with amenorrhoea. Cross-hatched area indicates range of response in control group. ■ indicates patients who also demonstrated FSH release after oestrogen provocation test.

oestrogen provocation, indicating a lesion in the hypothalamus or above, whereas evidently in some of the patients with post-pill or psychogenic amenorrhoea normal hypothalamic function existed.

The results of tests can be invalidated by elevated levels of progesterone, as found in the luteal phase of the menstrual cycle, when no positive feedback releases of LH in response to oestradiol can be induced in normal women. Similarly women with elevated gonadotrophin levels, as found in the premature menopause, after the normal menopause or with primary ovarian defects, show no positive release of LH after oestradiol, but in the above groups the presence of elevated gonadotrophin levels with low endogenous oestrogens must indicate an intact hypothalamic-pituitary axis (authors' unpublished work).

It would seem that apart from being able to predict clomiphene responsiveness from studying the gonadotrophin release after an oestrogen provocation test (Shaw *et al.*, 1975), the procedure is also useful in helping to define the functional abnormality causing the amenorrhoea. A further application which we are investigating is the use of the test in the localization of organic diseases in the region of the hypothalamus.

## ACKNOWLEDGEMENTS

This work was supported by a grant from the Medical Research Council. Our thanks to Dr. W. Bogie (Hoechst Pharmaceuticals) for supplying the LH-RH and to the technicians of the Department of Clinical Endocrinology who have so expertly performed the various assays.

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# THE RESISTANT OVARY SYNDROME

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## Summary

A patient is described with primary amenorrhoea, raised FSH and LH levels and ovaries containing many primary follicles. These features were characteristic of the "resistant ovary syndrome". After ovarian wedge resection the patient began to menstruate.

JONES and Moraes Ruehsen (1969) were the first to report three patients with primary amenorrhoea associated with failure of apparently normal ovaries to respond to gonadotrophin stimulation. Reports by Starup *et al.* (1971) and van Campenhout *et al.* (1972) appear to confirm the existence of this syndrome and its rarity. We record here a further case with many similar features but one important difference.

## CASE REPORT

The patient was first seen in another city at the age of 17 because of failure to menstruate. She was of normal height and secondary sexual development. Examination under anaesthesia showed a normal vulva and vagina and a normal-sized uterus. Laparoscopy revealed rather elongated ovaries, smooth in appearance and without evidence of follicular development. Chromosome analysis showed a normal female karyotype. Urinary oestrogen assays gave a value of 25 $\mu$g./l. Following the administration of exogenous oestrogens, withdrawal bleeding occurred.

The significant hormone assays were those for FSH and LH. The first assay showed urinary FSH levels of 100 IU./l. and LH levels of 370 IU./l., comparable with those of the post-menopausal age group. Two samples of blood were assayed for plasma LH. The first reading was 39·2 mIU./ml., a value corresponding to a point high on the mid-cycle peak; the second reading was 54·5 mIU./ml., well above the upper limit of normal even for the peak of a possible LH surge.

The patient moved to London and was 22 years old when seen by one of us (C.J.D.). There had still been no spontaneous menstruation and she was anxious to know if childbearing were possible. Although the evidence of the FSH and LH assays strongly supported the diagnosis of a premature menopause, it was decided to put matters beyond doubt by ovarian biopsy. At laparotomy the uterus was normal in appearance and the ovaries normal in size but rather smooth in appearance. A wedge was resected from each, the histology of which is reported later.

Six weeks after the ovarian biopsy the patient had a dark brown loss of blood per vaginam. Two weeks later (25th February) she had a normal period. Oestrogen assay eight days after this second loss of blood showed a urinary oestrogen level (Brown *et al.*, 1968) of 82 $\mu$g./24 hours (volume 1040 ml.); on the same sample a urinary FSH value of 208 IU./24 hours and an

LH value of 91·5 IU./24 hours were obtained by double antibody radioimmunoassay. Subsequently she menstruated on 17th April, 10th June, 4th July and 13th August, but no information about possible associated ovulation is available. Following the August period, 24-hour urine samples were collected weekly for FSH and LH assay and vaginal smears were taken on alternate days. Unfortunately the patient did not menstruate again and when seen in December was still amenorrhoeic. Vaginal smears showed no evidence of ovulation. Twenty-four hour urinary FSH and LH values were respectively as follows: 81·8 and 35·0 IU./24 hours on 2nd to 3rd September (urine volume 730 ml.); 125·4 and 53·8 IU./24 hours on 8th to 9th September (urine volume 1280 ml.); and 169·6 and 76·3 IU./24 hours on 19th to 20th September (urine volume 1060 ml.).

### *Histology*

The wedges from right and left ovaries measured approximately 10 mm. × 4 mm., with a maximum thickness of 4 mm. The superficial stroma below the surface epithelium was densely fibrosed. Deep to this was normal ovarian stroma with a plentiful supply of primordial follicles (Fig. 1). There were atretic follicles in various stages of evolution in both ovaries. Several, including the one depicted (Fig. 2) from the right ovary, showed well developed luteinization of the theca cells. An apparently fully developed corpus fibrosum was present in the left ovary (Fig. 3).

## COMMENT

Table I summarizes the features shown by our patient and the five others with the resistant ovary syndrome reported in the literature. The gonadotrophin levels are of the greatest interest and therefore are given in greater detail. Jones and Moraes Ruehsen (1969) merely report elevation of urinary total gonadotrophins, but radioimmunoassays on serum showed elevation of both FSH and LH levels. Starup *et al.* (1971) reported that total urinary gonadotrophins measured several times by the method of Johnsen *et al.* (1958) ranged from 82 MUU./day to 320 MUU./day which they merely comment upon as raised. Van Campenhout *et al.* (1972) reported total urinary gonadotrophins between 100 and 200 mouse units/day (2nd IRP-HMG);

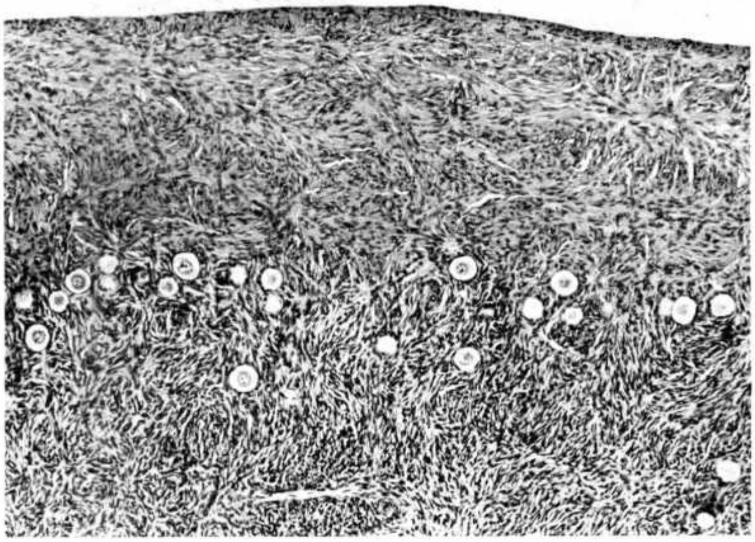

FIG. 1

Primary follicles in plentiful supply beneath fibrosed cortical stroma which contrast well with normal ovarian stroma at a deeper level (×88).

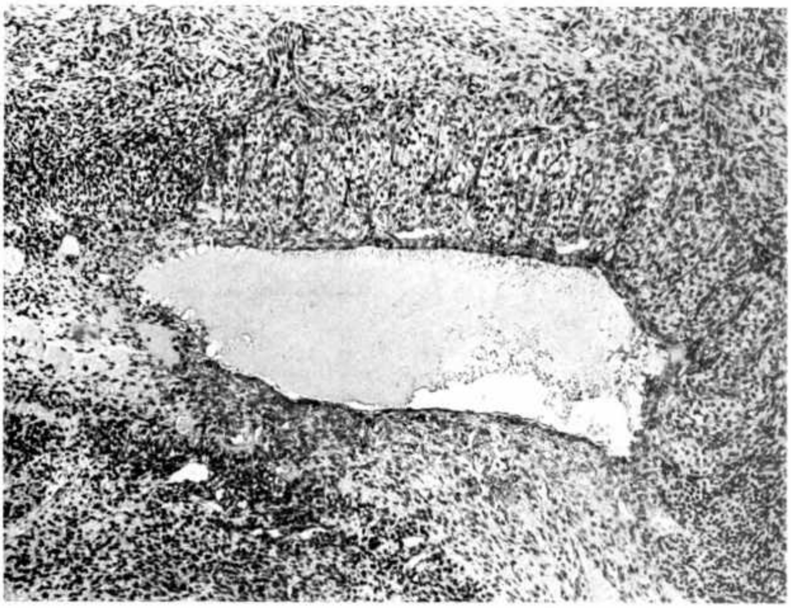

FIG. 2

An atretic follicle showing well-developed luteinization of theca cells (×88).

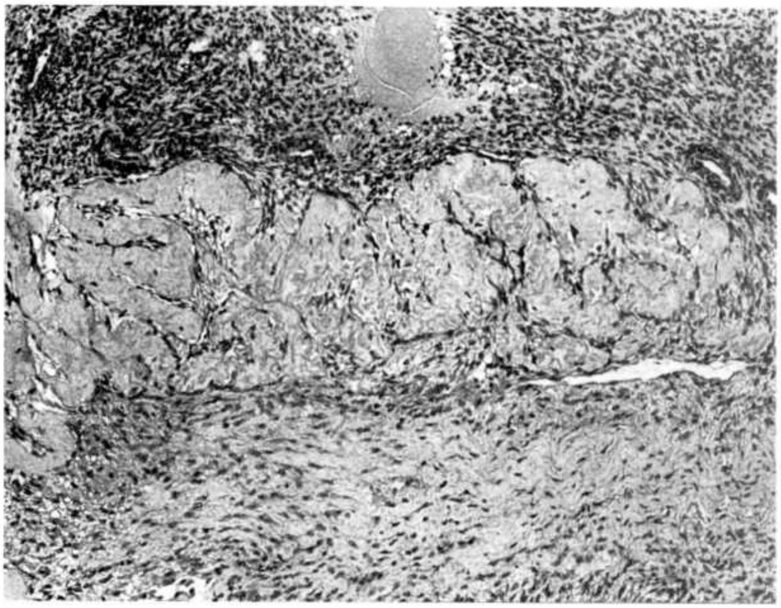

FIG. 3

An apparently well-developed corpus fibrosum (×88).

TABLE I

*Features shown by patients with resistant ovary syndrome*

| Author | Secondary development | Hormone withdrawal bleeding | Oestrogen assay | FSH/LH levels | Ovarian histology | Response to ovarian stimulation |
|---|---|---|---|---|---|---|
| Jones and Moraes Ruehsen (1969) (Three cases) | Normal | + | Between 5–10 μg./24 hours | High | Primary follicles in early development stage. Luteinized "stroma" | Slight to very high doses |
| Starup *et al.* (1971) | Below normal | + | Less than 2 μg./24 hours | High | Primary follicles, a few to antrum stage | None |
| Van Campenhout *et al.* (1972) | Below normal | + | 1·2 μg./24 hours | High | Primary follicles in early development stage. Luteinized theca | Slight to high doses |
| Our patient | Normal | + | (*a*) 25 μg./l.<br>(*b*) 82 μg./24 hours | High | Primary follicles and old cystic follicles. Luteinized theca. Old corpus fibrosum | — |
| | | | (*a*) 39 | Details of gonadotrophin levels are given in the text | | |

(*a*) Pre-operative. (*b*) Post-operative.

they quoted normal values as being between 10 and 50 mouse units/day; radioimmunoassay of LH in plasma showed values of 65, 90 and 70 mIU./m. (normal 4 to 36 mIU./ml.). Values obtained both before and after ovarian surgery in our patient were in the postmenopausal range.

Our patient was the only one to show a return of menstruation after biopsy of the ovaries. Her degree of follicular development and luteinization was greater than that found in others and the apparent corpus fibrosum suggesting ovulation at some earlier time cannot be explained. The greater degree of follicular development might well account for the relatively higher level of oestrogen excretion before and after operation. It is extremely puzzling, however, to have found a urinary FSH level of 208 IU/24 hours on the same day as the urinary oestrogen level of 82 μg./24 hours was obtained.

A diagnosis of resistant ovary syndrome is supported by high gonadotrophin levels in the presence of ovaries filled with follicles which are either not responding or doing so only slightly. Further evidence of ovarian resistance is found in the fact that attempts by the other authors listed in Table I to stimulate ovulation with high doses of clomiphene or gonadotrophins produced little or no response. The response to clomiphene and gonadotrophins was not assessed in our patient but it would appear reasonable to have made a diagnosis of resistant ovary syndrome although the degree of resistance was possibly less than in other reported cases. *Secondary* amenorrhoea due to the resistant ovary syndrome has been reported (Starup *et al.*, 1971, cases 2 and 3; Zourlas and Comninos, 1971), further suggesting that degrees within the syndrome permit it to present with slightly different clinical features.

It seems probable that the resistant ovary syndrome is a very rare cause of primary amenorrhoea. Only one example—that described here—was encountered in 117 patients with primary amenorrhoea who were fully investigated at Chelsea Hospital for Women in the five years between 1969 and 1973. The occurrence of even the infrequent case, however, along with the possibly more frequent occurrence of the syndrome in association with secondary amenorrhoea, raises the question of the reliance we can put upon FSH and LH values raised to postmenopausal levels in such cases. To assume that these high gonadotrophin results indicate a premature menopause without laparoscopic examination of the ovaries and perhaps biopsy for confirmation will mean that the occasional example of ovarian resistance will be overlooked. Unless there are other clear-cut clinical features supporting the diagnosis of a premature menopause (such as the features of gonadal dysgenesis, for example), ovarian biopsy should be considered before giving a firm prognosis that irreversible ovarian failure has occurred and childbearing is out of the question.

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# CREATINE PHOSPHOKINASE (CPK) LEVELS IN PREGNANCY: A CASE REPORT AND A DISCUSSION OF THE VALUE OF CPK LEVELS IN THE PREDICTION OF POSSIBLE MALIGNANT HYPERPYREXIA

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## Summary

Creatine phosphokinase (CPK) levels during two consecutive pregnancies of a patient whose sister had died of malignant hyperpyrexia have been investigated. The results are compared with CPK levels of serum obtained from apparently normal people at similar stages of pregnancy. The rise of serum CPK occurring as a result of labour was much more pronounced in the malignant hyperpyrexia suspect.

THE susceptibility to anaesthetic agents, referred to as the malignant hyperpyrexia syndrome, is well defined clinically (Britt and Kalow, 1970), and the incidence of deaths attributable to anaesthesia in related patients suggests that susceptibility to malignant hyperpyrexia is related to an autosomal dominant gene with reduced penetrance (Isaacs and Barlow, 1970). The biochemical lesion related to these symptoms, however, although thought to be related to muscle metabolism (Harrison *et al.*, 1969; Ellis *et al.*, 1971; Tammisto and Airaksinen, 1966; Bennike and Jarnum, 1964), is not fully understood and treatment is usually unsuccessful, the mortality rate being about 50 per cent (Britt *et al.*, 1969).

Recent work has been aimed towards devising screening techniques to detect susceptibility in patients at risk. The rationale of these techniques is based on reports in the literature which indicate a pronounced increase in several serum enzymes and especially of creatine phosphokinase (CPK), within a few hours after the administration of certain anaesthetic agents (Ellis *et al.*, 1972; Denborough *et al.*, 1970).

Even in apparently normal members of families in which malignant hyperpyrexia has occurred, increased CPK values have been observed (Isaacs and Barlow, 1970; Larrard *et al.*, 1972; Parikh and Thompson, 1972; Kyei-Mensah *et al.*, 1971). This has led to the suggestion that CPK levels may be used to screen for susceptible patients (Denborough *et al.*, 1971), although absolute correlation between a raised

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CPK level and development of hyperpyrexia is not proven (Kyei-Mensah *et al.*, 1971). The value of such CPK estimations has, however, been criticized because serum CPK increases from causes which are not related to malignant hyperpyrexia and because the appropriate set of normal limits must be considered when interpreting the results (Ellis *et al.*, 1972). Moreover, this question of normal limits becomes even more important in pregnancy since the values of CPK to be expected during the course of a normal pregnancy are somewhat equivocal. For example, elevated CPK levels have been observed in late pregnancy, labour and the puerperium (Konttinen and Pyörälä, 1963; Dambrosio *et al.*, 1966), or only during the puerperium (Variati *et al.*, 1966), whilst no increase has been found in a small number of patients examined in the last trimester (Pearce *et al.*, 1964).

The aim of the present work, therefore, was firstly to investigate the CPK levels of a malignant hyperpyrexic suspect during her pregnancy and secondly to compare these levels with a range of CPK levels established in apparently normal pregnant individuals.

## CASE REPORT

In early 1970, Mrs. B., aged 25 years, was referred to Leeds Maternity Hospital early in her second pregnancy. Her sister had died of malignant hyperpyrexia associated with general anaesthesia (Kyei-Mensah *et al.*, 1971). The CPK levels of the patient and her family at this time are shown in Table I. At 38 weeks gestation a further CPK estimation was carried out. This value and subsequent levels during labour and the early puerperium are listed in Table II.

TABLE I

*Serum creatine phosphokinase (CPK) values in our patient's family*

| | CPK (IU/1 at 37 °C.) (Normal range 0 to 86 IU/1) |
|---|---|
| Father | 27 |
| Mother | 305 |
| First brother | 462 |
| Second brother | 381 |
| Third brother | 62 |
| Daughter | 108 |
| Patient | 143 |

TABLE II

*Serum CPK levels during and after our patient's second pregnancy*

| Date | CPK levels (IU/1 at 37 °C.) | |
|---|---|---|
| | Patient | Normal range |
| Early 1970 | 143 | 0 to 86 |
| Thirty-eight weeks gestation | 755 | 0 to 103 |
| Immediately after delivery | 656 | 0 to 103 |
| Eight days after delivery | 403 | 0 to 103 |

Duration of labour was 16 hours 40 minutes.

The patient's labour proved uneventful and resulted in the normal spontaneous delivery of a second healthy child. Analgesia was provided by pethidine and Entonox.

No further investigations were carried out until this patient reported for booking during her third pregnancy at approximately 30 weeks. Table III shows the results of the CPK estimations made during that pregnancy. Labour again progressed uneventfully with the delivery of a normal child. Unfortunately, it has proved impossible to obtain further samples from this patient.

## METHODS OF ASSAY

The CPK assays carried out during the first and second pregnancy of Mrs. B. were carried out at 30 °C. using the Calbiochem reagent kits. The normal range for non-pregnant adults, quoted by the manufacturers' was 0 to 60 IU/1 at 30 °C. The CPK assays during the patient's third pregnancy were carried out at 37 °C. using

TABLE III

*Serum CPK levels during and after our patient's third pregnancy*

| Time | CPK level (IU/1 at 37 °C.) |
|---|---|
| 30 weeks gestation | 890 |
| 31 weeks gestation | 850 |
| 37 weeks gestation | 1300 |
| In labour (duration of labour 12 hours) | |
| 12 hours before delivery | 1740 |
| 5 hours before delivery | 1055 |
| 1½ hours before delivery | 1540 |
| 9 hours after delivery | 1420 |
| 3 days after delivery | 870 |

TABLE IV

*Normal CPK levels during pregnancy and early puerperium. SVD=spontaneous vertex delivery*

| Patient | Age (years) | Parity | Duration of labour (hours/minutes) | Type of delivery | CPK levels (IU/1 at 37 °C.) | | |
|---|---|---|---|---|---|---|---|
| | | | | | At onset of first stage | 18 to 24 hours after delivery | 7 to 8 days after delivery |
| 1 | 24 | 0+0 | 20·00 | Forceps | 39 | 447 | 63 |
| 2 | 21 | 0+0 | 11·58 | SVD | 34 | 194 | 102 |
| 3 | 24 | 0+0 | 5·13 | SVD | 34 | 173 | 115 |
| 4 | 23 | 0+4 | 8·00 | SVD | 42 | 141 | 68 |
| 5 | 22 | 0+0 | 15·83 | Forceps | 98 | 214 | 93 |
| 6 | 20 | 0+0 | 2·83 | SVD | 24 | 53 | 39 |
| 7 | 32 | 2+0 | 2·13 | SVD | 38 | 96 | 29 |
| 8 | 20 | 0+0 | 8·50 | SVD | 29 | 216 | 44 |
| 9 | 22 | 0+0 | 5·83 | SVD | 91 | 552 | 62 |
| 10 | 25 | 0+2 | 5·33 | SVD | 49 | 263 | 34 |
| 11 | 21 | 0+0 | 8·62 | SVD | 49 | 825 | 59 |
| 12 | 24 | 0+0 | 8·33 | Breech | 49 | 197 | 184 |
| 13 | 21 | 0+0 | 17·00 | SVD | 151 | 241 | 73 |
| 14 | 23 | 0+0 | 19·66 | Forceps | 329 | 637 | 348 |
| 15 | 25 | 0+1 | 15·42 | Forceps | 230 | 825 | 39 |
| Means | 23·1 | | 10·28 (SD = 5·94) | | 85·73 | 338·3 | 90·13 |

Eskalab reagent kits, the normal range quoted by the manufacturers in this case being up to 130 IU/1 at 37 °C. The principle of both these assays is the same (Oliver, 1955). In order that comparisons between results in the second and third pregnancies could be compared, all normal ranges and CPK levels originally measured at 30 °C. have been multiplied by 1·72 to give IU/1 at 37 °C.

## RESULTS

The data relevant to the last two pregnancies of Mrs. B. are presented in Tables I to III.

In order to obtain control CPK values two groups of patients were studied; the first was a random sample of 18 patients attending the antenatal clinic in the first trimester of pregnancy. A blood specimen was taken from each of these patients, without constriction of the arm. The second group were patients presenting in early labour. Blood was obtained at this time, within 24 hours of delivery and approximately seven days after delivery.

The CPK values in the first control group measured at 37 °C. ranged from 19 to 92 IU/1, the mean being 44 IU/1. The results of the CPK estimation and some details of labour in the second group of patients are presented in Table IV.

## DISCUSSION

Early in her second pregnancy our patient's CPK level was 143 IU/1 (Table I) which was high when compared to the range of values in non-pregnant adults (0 to 86 IU/1) and the range of values (7 to 81 IU/1 at 37 °C.) in women tested in the first trimester of pregnancy. Consequently, it indicated her potential susceptibility to malignant hyperpyrexia. The high CPK levels were maintained throughout labour and the early puerperium (Tables II and III).

The CPK levels in the second control group (Table IV) showed that normal pregnant women have a marginally significant increase ($0{\cdot}10 > P > 0{\cdot}05$) in the CPK level to 85·6 IU/1 in early labour compared with the mean level in the first trimester (44 IU/1) but this may well be related to early uterine activity (patients 13, 14 and 15 had all been experiencing contractions for some time prior to blood sampling). The mean CPK level 24 hours after delivery (338 IU/1) was significantly raised ($P < 0{\cdot}001$; paired "t" test) compared with pre-labour samples. The rise in CPK values correlated to some extent with the length of labour suggesting it was related to muscular effort by the uterus; for patients who had a spontaneous vertex delivery (mean duration of labour 7·5 hours), the CPK level was 275 IU/l compared to a level of 531 IU/l for patients who had a forceps delivery (mean duration of labour 17·73 hours). The high CPK levels noted in our patient's third pregnancy, with a labour of 12 hours, were therefore unlikely to be due to excessive muscular effort.

In the majority of cases CPK levels, seven to eight days post partum, are again the same as those observed in early labour. Two patients (Nos. 12 and 14) in the control group still showed markedly elevated CPK levels at 7 to 8 days post partum. Two months after delivery their CPK levels were respectively 52 and 108 IU/l and within the normal non-pregnant range.

Ellis *et al.* (1972) pointed out that the value of CPK levels as a screen for susceptibility to anaesthetic agents is limited because they can be increased by exercise and by diseases not related to malignant hyperpyrexia. The changes in CPK levels during pregnancy introduce an additional complication in screening for potential cases of malignant hyperpyrexia. However, our patient who had a family history of malignant hyperpyrexia had CPK levels which were raised throughout pregnancy and for several days after delivery.

The data presented suggest that a CPK level of greater than 110 IU/l, in the first trimester or in early labour, indicates probable susceptibility to malignant hyperplexia and steps should be taken to avoid anaesthetic agents such as suxamethonium and halothane which might precipitate the syndrome. For these patients conduction analgesia would be safer.

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April 1975. Vol. 82. pp. 350–352.

# BOOK REVIEWS

**Oral Contraceptives and High Blood Pressure.** Edited by MELVIN J. FREGLY and MARILYN S. FREGLY. Pp. xiii+375. The Dolphin Press, Gainesville, Florida. Price: $12.50.

THIS book, which is a series of papers presented at a symposium held at the College of Medicine of the University of Florida in March 1973, still remains, despite the considerable delay before the results have been circulated to a wider public, the best collected source of information on the subject. While it is difficult to see how some of the papers fit into the presentation (for example, the excellent work of Muirhead and his colleagues on the antihypertensive action of the renal medulla) there are many excellent reports on the occurrence of hypertension in women on oral contraceptives touching both on epidemiology and very heavily on the renin-angiotensin system and aldosterone. There are no conclusive answers to be found but it does enable the reader to see which of the measurements of renin activity, substrate, plasma aldosterone and sodium and water balance are likely to be valid. There is wide confirmation of the reduction of renin as such during the administration of oral contraceptives with a marked rise in renin substrate leading to increased plasma renin activity. No differences emerged which explained why some women with exactly the same change in plasma renin activity and sodium balance developed hypertension while others did not. The shadowy role of the steroids, aldosterone, progesterone and the oestrogens, was not diminished and it is obvious that much work needs to be done. Since ten of the papers concerned the rat, it was necessary to wonder whether this animal did in fact provide a good model for the human female; opinions might be divided on this but the overall contribution from this source was disappointing. This is one of the situations where the social situation provides subjects for investigation where to this reviewer medical ethics would press for deeper investigation and not just condone it. The book is well produced and, at $12.50, is reasonable by modern standards. Clinicians as well as laboratory research workers will find information of real value, and, in view of the widespread use of oral contraceptives, it is a subject on which much of the medical profession needs to be kept well informed.

*W. S. Peart*

**Handbook of Physiology. Section 7 Endocrinology Volume II. Female Reproductive System Part 1 and Part 2.** Executive Editor: STEPHEN R. GEIGER. Pp. vii+653 (Part 1); 375 (Part 2) with 333 figs. and 51 tables (Part 1); 240 figs. and 57 tables (Part 2). The William & Wilkins Company. Price: $44.50 (Part 1); $25.00 (Part 2).

THESE two volumes are literary isomers and should be taken together. The attempt by the American Physiological Society to produce a series of texts covering all aspects of human physiology is an ambitious enterprise. In the endocrinology section alone, these two large volumes on the female reproductive system comprise more than a thousand pages of double column text in 50 separate sections written by 66 authors, almost all American. This is the most comprehensive text on female endocrinology yet written in the English language and deserves the attention of all physiologists, endocrinologists and gynaecologists. The individual chapters are grouped together under broad headings but the basis of the division of material between the two books is not obvious, other than that the ovary appears in Part 1 and pregnancy in Part 2. The figures are clear and simple, the layout excellent and the construction and usage uniform. There has clearly been a strong editorial hand, both on the production and on the editorial side. The authors have evidently had firm instructions to write proper reviews and not to produce linked summaries of references. The writing of a book by a large number of authors is at best a hazardous enterprise. The prospect is a little brighter when it is organized as a book *ad initio* and is produced by selected writers rather than as the written record of a meeting. Roy Greep has done well in his selection. Almost every well known American name in reproductive endocrinology appears. It speaks volumes for his authority and skill that he has been able to impose uniformity of format, minimal overlapping and a generally high level of scientific writing on such a heterogeneous collection of eminent people. No reviewer can pretend to have read the whole of a work of this size (although the Editor did give me six months to do the job), nor is it possible to make mention of each of the 50 chapters. In order to give something of the flavour of the book I propose to pick out a few sections, both good and bad. The section on endocrine control of egg implantation by Alexandre Psychoyos is one of the few by a non-American author in Part 1 Most of the work in this field involves animal experimentation and has appeared in non-clinical publications; it will therefore be strange to many gynaecologists. I found his guided tour through these foreign paths clear and interesting; he has the gift of conveying the sense of the biological universality underlying the rat data. The electron micrographs showing the first contact between the microvilli of the endometrium and the trophoblast are fascinating. Two of the gynaecologists appearing in Part 2 also do well. Although the section on the passage of hormones through the placenta, by John Josimovich, is short (only 7 pages including references) it manages to pack in a good deal of information on a subject of prime interest in obstetric practice. Ken Ryan on steroid hormones in mammalian pregnancy is equally terse; not surprisingly this master of obstetric endocrinology, reviewing a subject to which he has made major contributions, provides an object lesson in scientific writing. In Part 1 Richard Landau makes a coherent story of the many metabolic effects of progesterone. Nobody not versed in the subject would guess from this chapter that most of our knowledge is derived from his own experimental work. He stays well within his competence and skirts the controversial area of the progesterone block in pregnancy. Of course this is an issue which Heap and his collaborators could not avoid in their chapter on the hormonal maintenance of pregnancy. It must have been very tempting to come down in favour of progesterone withdrawal as a cause for the onset of labour, particularly as they have produced some of the animal data supporting this view. It is very fair to conclude, as they did, that the view that parturition is initiated by a sudden fall in circulating levels of progesterone is not supported by work on those species in which the placenta secretes appreciable amounts of progesterone. The dangers of scholasticism are ever present in a book of this kind. At times the thread of the story is lost in the chapter on the physiology of progestins by Goldman and Zarrow and they were at some pains to work in 252 references for 19 pages of print. The chapter on the mechanism of action of progesterone by O'Malley and Strott is disappointing. The discoveries on the intracellular actions of oestrogens have opened broad avenues in reproductive endocrinology. The view down the progesterone avenue should be full of exciting possibilities but none of this comes through. It is a near miss for they have uncovered an animal model (avidin synthesis by the chick oviduct), which provides an admirable means of exploring the role of progesterone in cell biochemistry. Perhaps it is too soon to see the relevance of rat deciduoma or chick oviduct to human physiology, but I had hoped for more on what progesterone does inside the cells of the endometrium and the myometrium, and how this reflects on the events of the menstrual cycle and pregnancy. This series must be heavily subsidised by the American Physiological Society. At $69.50 the price is well below that of comparable American publications. At all events this brings a major publication within the reach of all medical libraries. It will be a standard reference work for years to come and must be acquired. For those still able to buy scientific books for themselves, this tops the list.

*A. Klopper*

**Immunology in Obstetrics and Gynaecology.** Edited by A. Centaro and N. Carretti. Pp. 320. Excerpta Medica, Amsterdam, 1974. Price: US $40.40.

The First International Congress of Immunology in Obstetrics and Gynaecology took place in June 1973 but only now do its proceedings appear in the form of this book. Although much of the work presented was new at the time many of the authors have, in the intervening period, either published the same, or slightly amplified, data elsewhere or described them at subsequent conferences. For those working in the field, therefore, the book contains little that is new. The aim of the Congress, and thus the book, was to make gynaecologists and obstetricians aware that immunological problems exist in infertility, feto-maternal relations, oncology and endocrinology. This book, however, is only occasionally of help to those with no specialized knowledge who are attempting to gain insight into these problems. One has the right to expect more of real and lasting value in a publication costing nearly £18. Congress proceedings can be valuable if produced quickly and relatively inexpensively: the Proceedings of the Second International Congress of Immunology were published within six months and those of the Fifth International Congress of the Transplantation Society are to cost one-third of the price of this volume, despite the fact that these congresses were much larger than this one. One also suspects that some authors rely on congress proceedings to provide them with "a publication" the data in which they have every intention of also submitting elsewhere. If this is so, it is to be deprecated. This book may, in conclusion, be of interest to those who attended the congress, but has little to commend it to anyone else.

*G. M. Stirrat*

**Urodynamics. Upper and Lower Urinary Tract.** Edited by W. Lutzeyer and H. Melchior. Pp. xii+344 with 209 figs. Springer-Verlag, Berlin, Heidelberg, 1973. Price: US $52.50.

This is an excellent but expensive book which is rather more technical than clinical in its orientation and is mainly directed to the urologist, but should be of considerable value to the gynaecologist interested in the vesicourethral function. It comprises some 45 papers delivered at the first International Congress of Urodynamics by the leading research workers from Europe and the United States of America and covers most of the current concepts on the physiology and pharmacodynamics of the urinary tract. About half the contributions are focused on ureteric function in which the mechanisms and regulation of peristalsis, together with the influence of drugs and obstruction are considered in detail with a particularly interesting, well written review and discussion by Kiil. The second half of the book studies vesicourethral dynamics covering mechanisms of normal micturition, continence, incontinence and voiding and is put into clinical perspective by an excellent contribution by Turner Warwick. Although many of the articles are brief and often abstruse in their description of the apparatus and the variety of techniques for studying pressure and flow, there are several fascinating clear surveys of current concepts of urodynamics. In the past, the behaviour of the urinary tract has been investigated by the cystoscope and on the X-ray screen, but this stimulating book shows that even more useful information can be collected by a variety of electronic probes, putting research in this field on a par with cardiopulmonary studies.

*J. Malvern*

**Heart Disease and Pregnancy.** By P. Szekely and L. Snaith. Pp. ix+217 with 55 figs. and 27 tables. Churchill Livingstone, Edinburgh, 1974. Price: £7.00.

This book is a welcome and long overdue British contribution to the literature on this subject. Tribute must be paid to such good cooperation between a cardiologist and an obstetrician over three decades. The format is good and makes for easy assimilation of the data which has obviously been painstakingly collated. The many illustrative electrocardiograms and X-rays have been carefully selected and are beautifully reproduced. The bibliography is most comprehensive. The authors' main interest is obviously rheumatic heart disease and this is reflected in the somewhat disproportionate number of chapters devoted to this topic. It is, however, surprising that over a thirty year period such small numbers of pregnant patients with cyanotic congenital heart disease were observed, either with or without palliative surgery. The authors' approach to this difficult and growing problem is perhaps too pessimistic and there must be a reservation about advising such patients to avoid pregnancy. The attitude to epidural analgesia and mechanical ventilation seems rather cautious and a surprising omission was the lack of comment on the effects of the contraceptive pill in patients with various forms of heart disease. This book is unreservedly recommended a place in postgraduate libraries and as mandatory reading for all cardiologists, obstetricians and interested general physicians, who are involved in the joint care of pregnant patients with heart disease.

*G. A. Batson*

## PUBLICATIONS RECEIVED BUT NOT REVIEWED

**Practical Obstetrics. A Short Textbook in English and Chinese for Students and Midwives.** By D. W. C. Chun and K. H. Lee. (Translated by H. P. Lau and K. H. Lee). Pp. xii+507, with figs. and tables. Hong Kong University Press, 1974.

**Mid-Trimester Pregnancy Terminations. A Comparison of Methods.** By A. Mehta, R. Talati, S. Mirchandani and J. Katrak. Pp. 13. India Fertility Research Programme Conference Paper.

**Life—Death—And Modern Medicine.** By Ian Donald. Pp. 24. Order of Christian Unity, 39 Victoria Street, London SW1. Price 20p.

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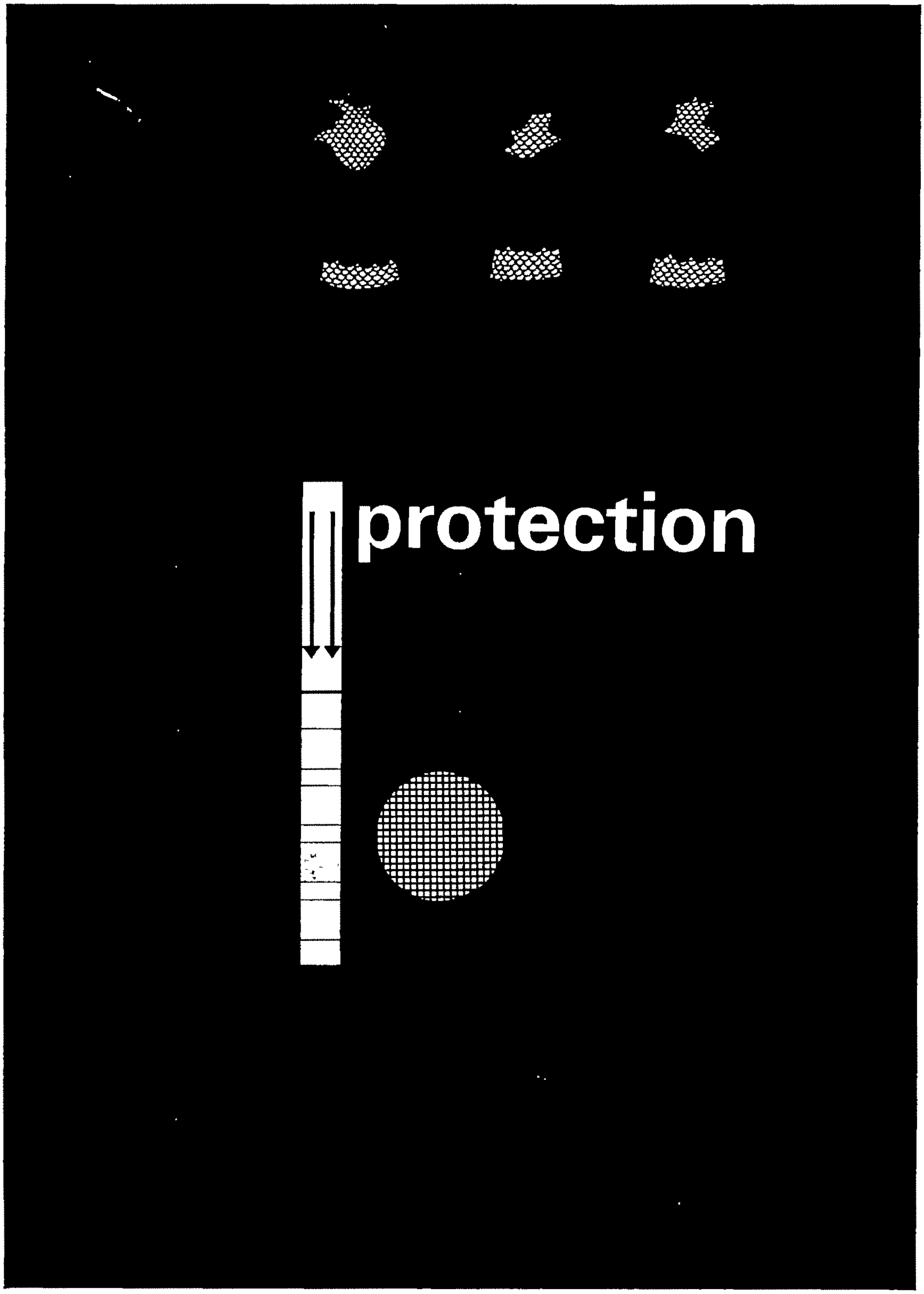
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Wherever possible, authors should express measurements in SI units and whenever such a measurement appears in the text of an article it should be followed in parentheses by the equivalent in traditional units. Tables and figures should be presented in SI units, conversion factors to traditional units being given as footnotes. Those seeking guidance should refer to their local laboratories or to an article by Professor D. N. Baron at page 509 of the *British Medical Journal* of 30th November, 1974.

## ABBREVIATIONS

These should be avoided as far as possible. "Per cent" should be used instead of the symbol "%" and such forms as T.B., R.B.C., B.P., P.E.T., A.P.H. eschewed. Dates should be given as 10th May, 1974 and *not* 10.V.74. The official rather than the proprietary names of drugs must be used.

The Journal convention is C for Celsius, m for metre(s), g for gramme(s), A for ampere(s), k for kelvin(s), cd for candela(s), m for mole(s), l for litre(s) and "/" for the divider "per". Submiltiples of units are formed by prefixes and authors should use d for deci or $10^{-1}$, c for centi or $10^{-2}$, m for milli or $10^{-3}$, μ for micro or $10^{-6}$ n for nano or $10^{-9}$, p for pico or $10^{-12}$, f for femto or $10^{-15}$ and a for atto or $10^{-18}$; for multiples the prefixes should be T for tera or $10^{12}$, G for giga or $10^{9}$ M for mega or $10^{6}$ k for kilo or $10^{3}$ h for hecto, or $10^{2}$ and da for deca or $10^{1}$. Thus it would be kg. for kilogramme(s), ml for millilitre(s), μg for microgramme(s), etc. Symbols should be typed without a full stop. In the writing of large numbers, groups of three figures should be separated by a space and not a comma (to avoid confusion with decimal points).

## REFERENCES

In the text the author's name should be given, with the date of publication and the list of author's quoted put at the end of the text in alphabetical order as in the Harvard Scheme: Author's name, initials, year of publication (in brackets), name of journal in full (*italics*), volume number (underlined), and page number. In the case of books the order is: Author's name, initials, year of publication, full title (*italics*) edition, publisher and place of publication, page, thus:

Brown, H. E., and Jones, B. K. (1961): *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **2**, 128.

Sampson, P. (1940): *Obstetrics for Midwives.* 2nd edition Fraser, London, p. 9.

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'. . . a clinically effective analgesic with a rapid onset of action . . . '[1]

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'The finding of a high incidence of side effects after pethidine is in keeping with other studies . . . emetic sequelae and dizziness being particularly troublesome . . . it was distinctly inferior to pentazocine [Fortral] in this respect.'[3]

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**eases the progress and pain of labour**

1. Obstet. Gynec. (1966) 28:224-232.
2. 'Pain' (1972) Churchill Livingstone, London. pp. 395-397.
3. Brit J. Anaesth. (1974) 46.365-372.

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1. Brit. Med. J., (1973), **4**, 64

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J.Obstet.Gynaec. Brit. Cwlth. 1972. **79.** 517.

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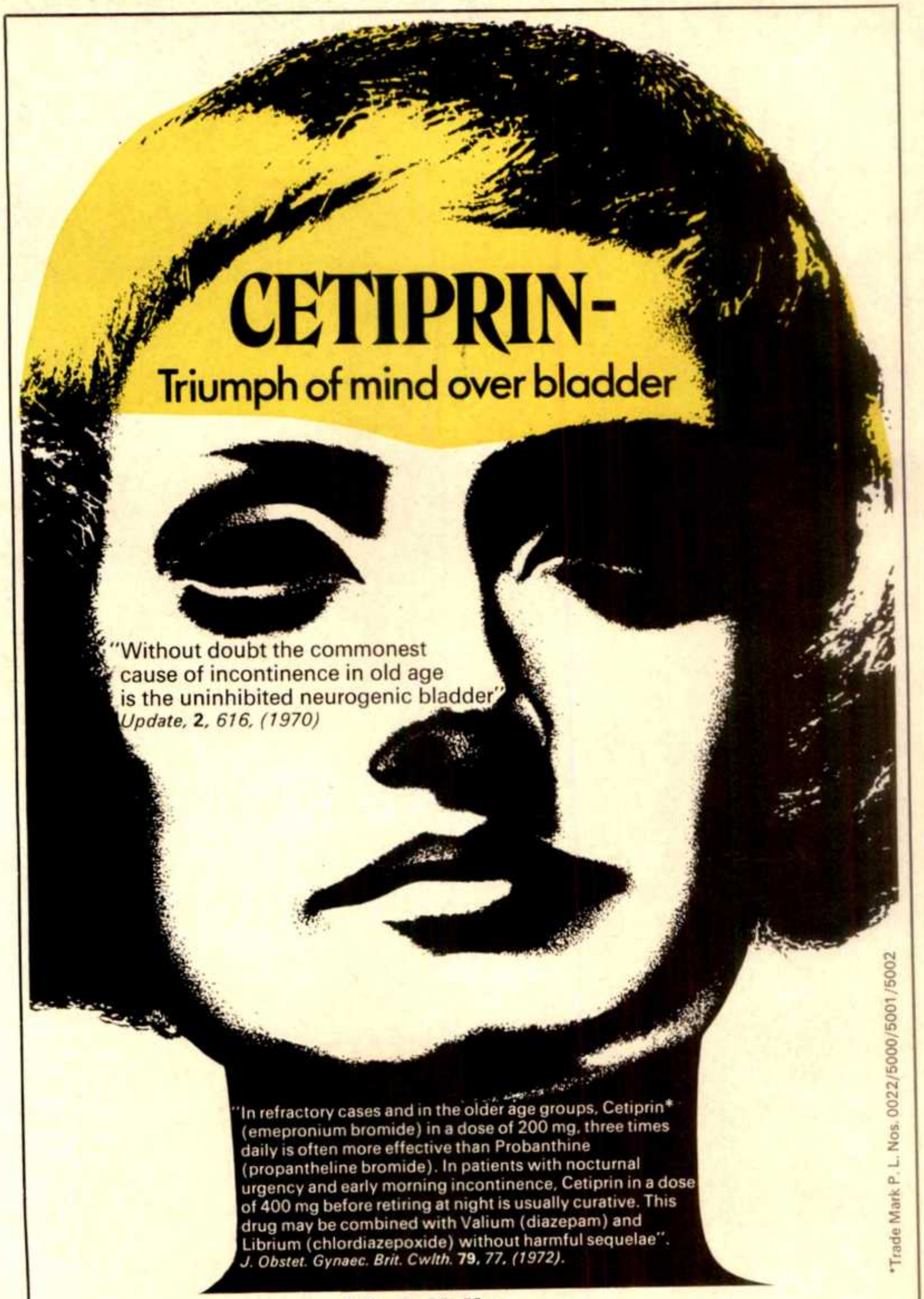

# CETIPRIN-
## Triumph of mind over bladder

"Without doubt the commonest cause of incontinence in old age is the uninhibited neurogenic bladder"
*Update*, **2**, *616, (1970)*

"In refractory cases and in the older age groups, Cetiprin* (emepronium bromide) in a dose of 200 mg, three times daily is often more effective than Probanthine (propantheline bromide). In patients with nocturnal urgency and early morning incontinence, Cetiprin in a dose of 400 mg before retiring at night is usually curative. This drug may be combined with Valium (diazepam) and Librium (chlordiazepoxide) without harmful sequelae".
*J. Obstet. Gynaec. Brit. Cwlth.* **79**, *77, (1972)*.

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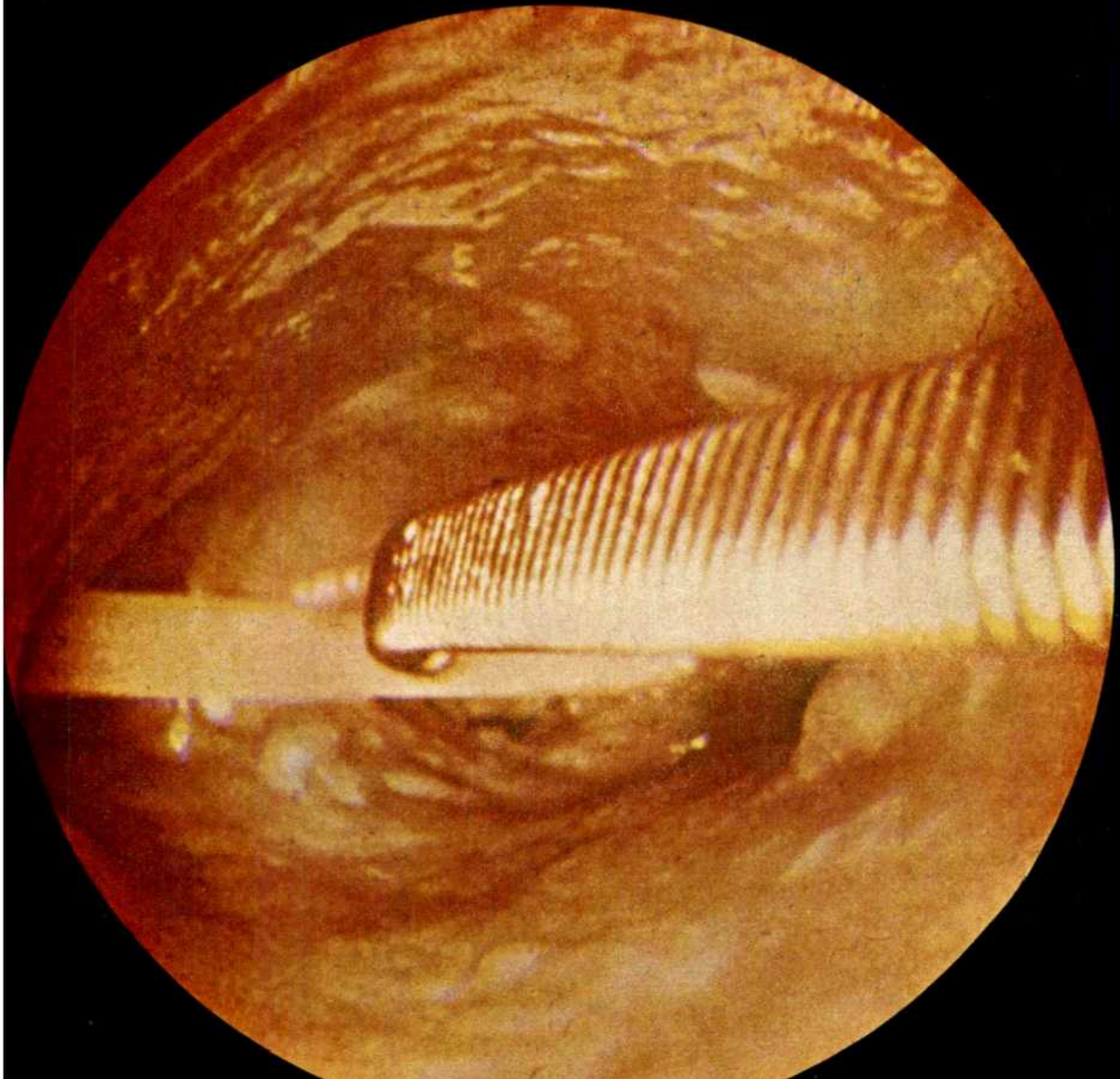


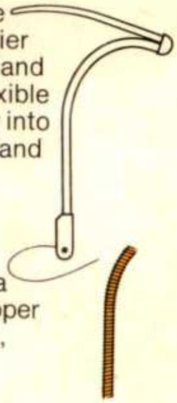
Gravigard in situ in
the uterine cavity

# **Gravigard** Contraceptive - the best alternative to the pill.

- Gravigard is the most advanced method of intrauterine contraception available and is only surpassed in efficiency by the combined pill.

| Method | Failure rates per 100 women years |
|---|---|
| No contraceptive[1] | 60 |
| Coitus interruptus[1] | 35 |
| Safe period[1] | 20 |
| Diaphragm[1] | 10 |
| Condom[1] | 10 |
| I.U.D.[2] | 5 |
| Progestogen-only pill[3] | 2·4 |
| **Gravigard**[3] | 1·1 |
| Combined pill[3] | 0·3 |

- Easy insertion is aided by the small size of the Gravigard carrier which also reduces discomfort and bleeding after insertion. The flexible 7 shape of Gravigard fits snugly into the uterus – so aiding retention and causing fewer expulsions.

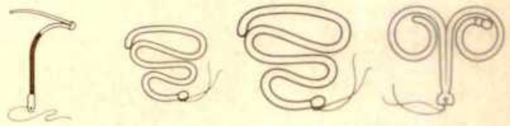


- The plastic 7 merely acts as a carrier for the contraceptive copper which exerts a non-mechanical, local action on the uterus.

- Gravigard is easily and virtually painlessly inserted – the introducer tube diameter of 3 mm. is the smallest of any I.U.D.

Lippes 5·4 mm. ● (approx)
Saf-T-Coil 4·5 mm. ●
Uterine sound 3·5 mm. ●
**Gravigard 3·0 mm.** ●

- Gravigard, in contrast to other I.U.D.s., has a remarkably low incidence of removal because of pain and bleeding.

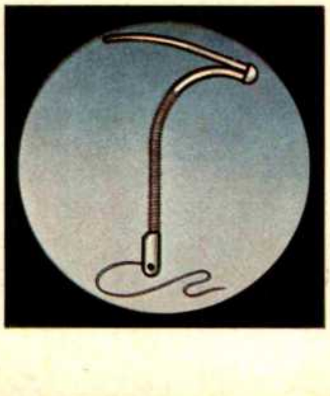


| **Gravigard**[2] | Lippes Loop C[4] | Lippes Loop D[4] | Saf-T-Coil[4] |
|---|---|---|---|
| 4·1 | 11·0 | 11·7 | 14·7 |

- Both multiparous and nulliparous women can be fitted with, and retain, Gravigard.

- When these advantages are combined with a reported pregnancy rate as low as 0·7/hundred women years[5] it's clear why Gravigard offers the best alternative to the pill.

**Gravigard**
Contraceptive



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Further information is available on request.

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[1] Human Reproduction and Society, Faber, 1974
[2] Brit. Med. J., 1974, **3**, 447–450
[3] Data on files of Searle Laboratories
[4] IPPF booklet "Intrauterine Contraception"
[5] Am. J. Obstet. Gynecol. 1973, **117**, 602

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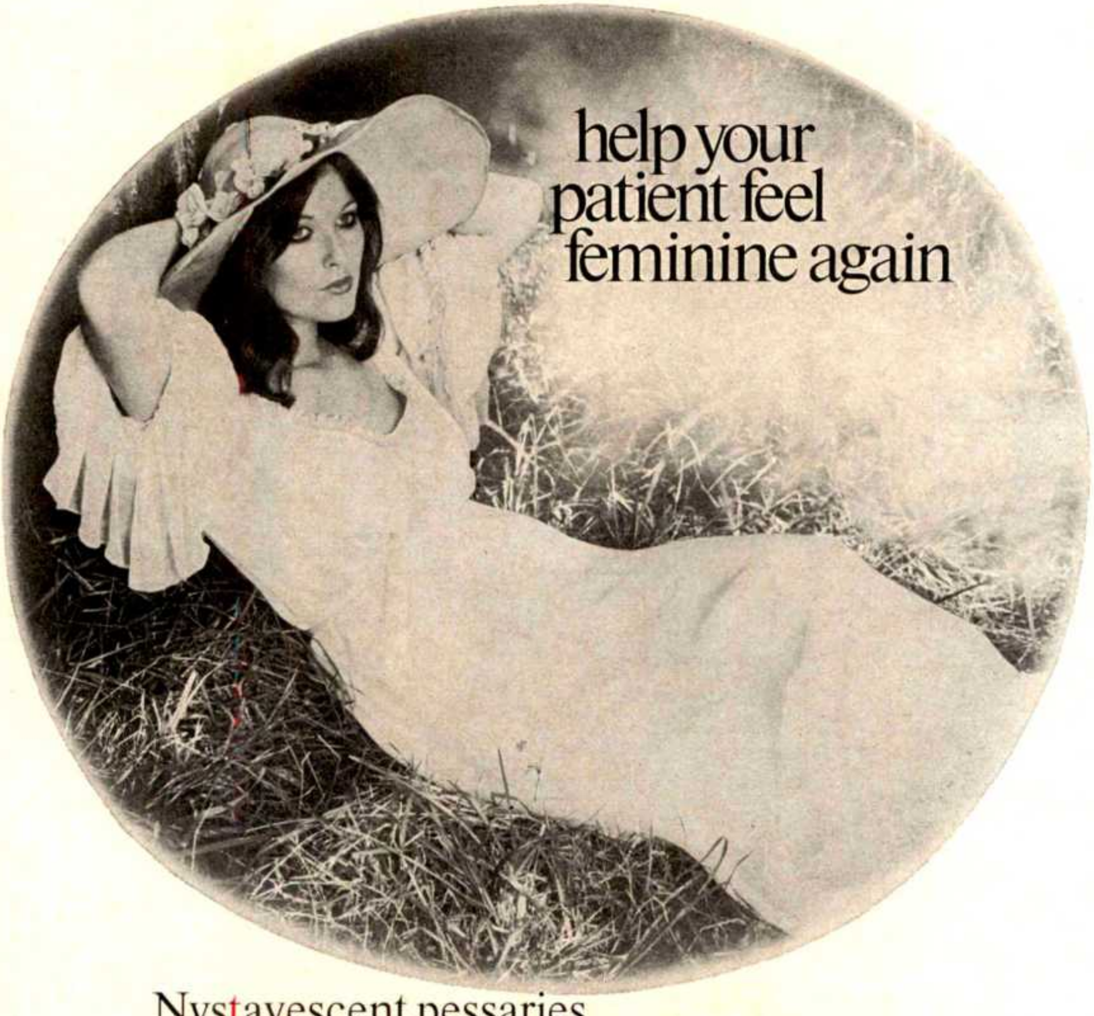
pessaries



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a new answer to the growing problem
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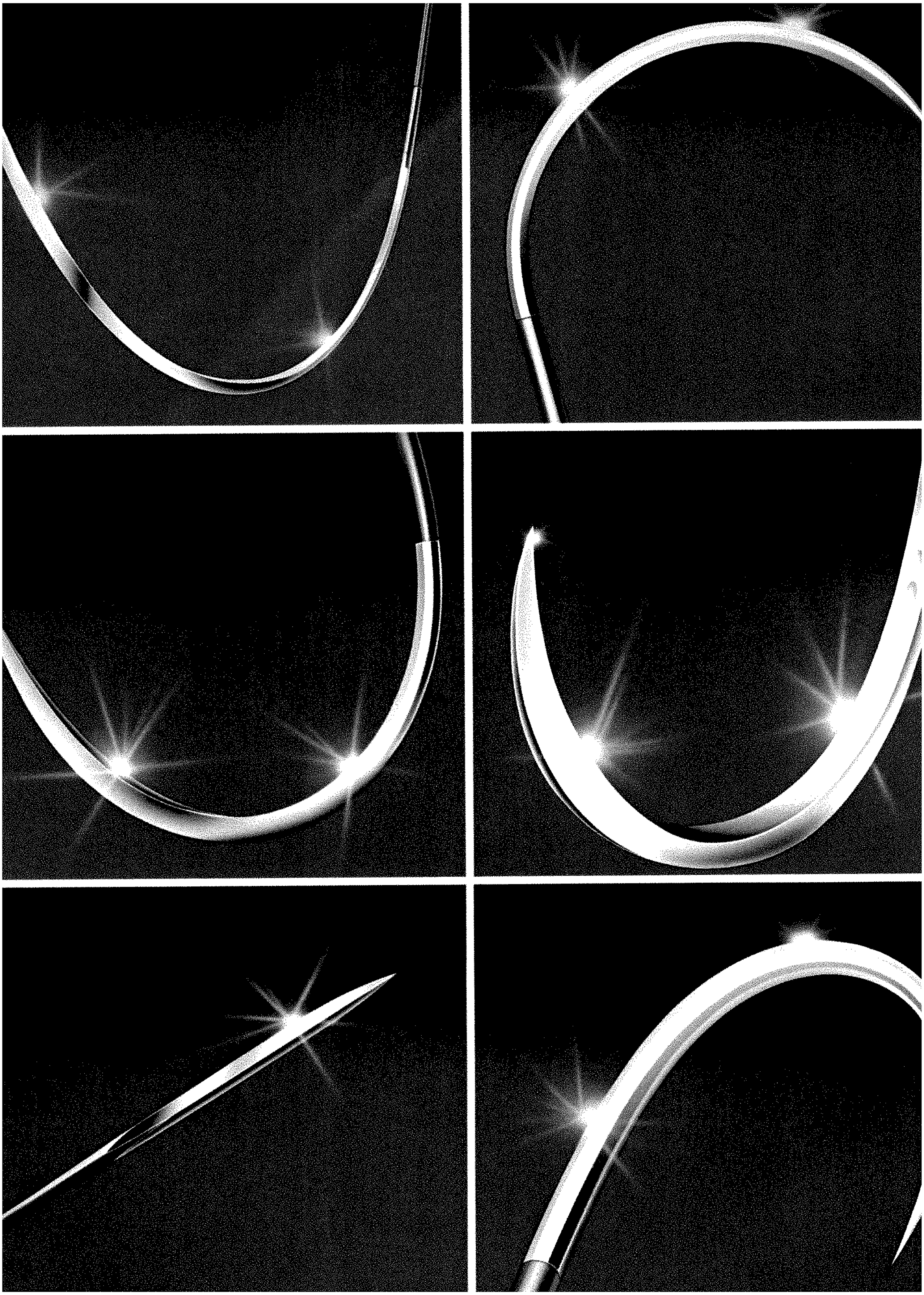

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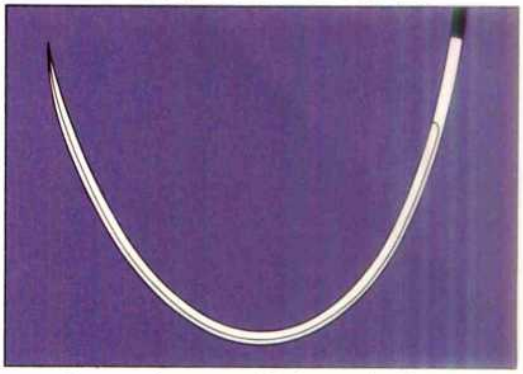

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A range of curved cutting needles attached to a choice of suture materials is available for those surgeons preferring the 'hand-held' technique for skin closure.

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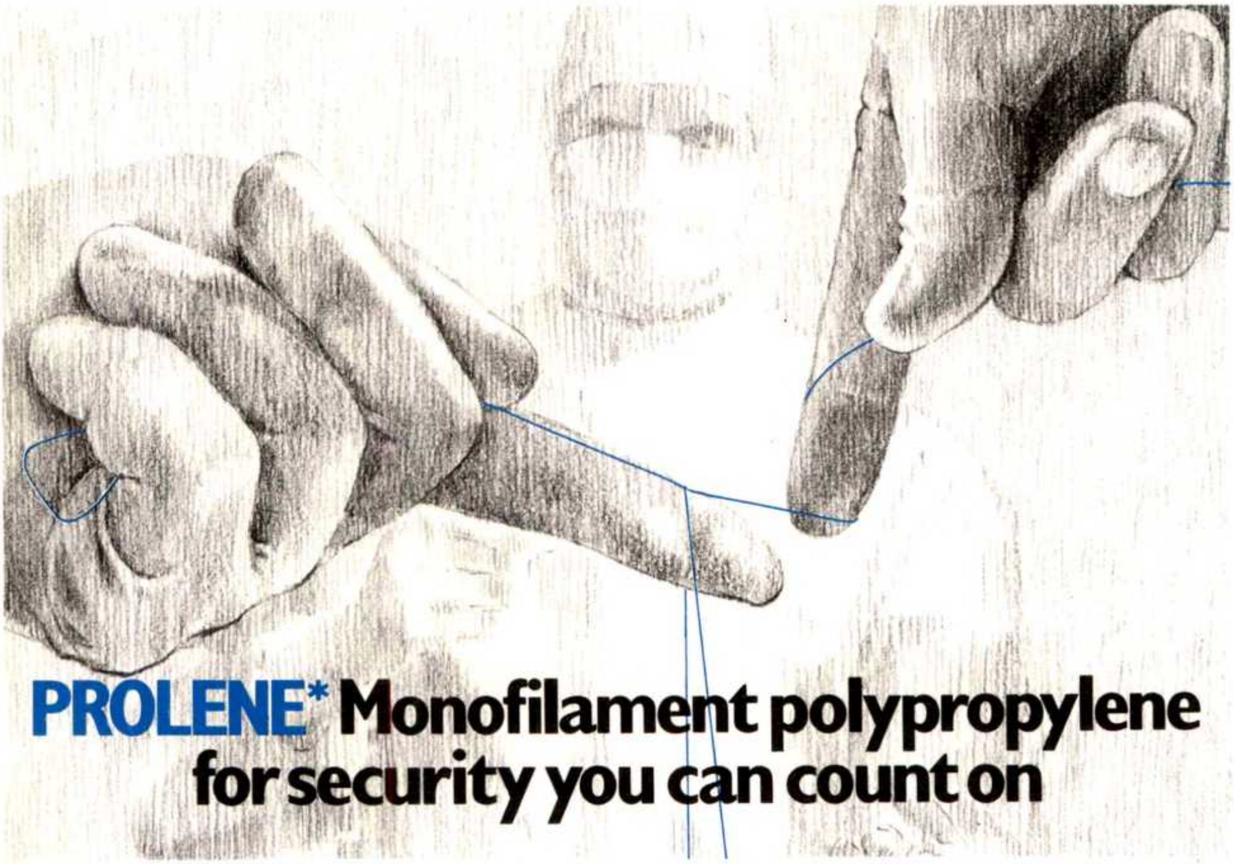

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When the reliability of a non-absorbable is required in a contaminated or potentially contaminated field many surgeons are now choosing PROLENE suture. Made from polypropylene, a material of relative biological inertness, monofilament PROLENE suture is not degraded by tissue enzymes and is virtually unaffected by the presence of infection.

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PROLENE suture is able to deform and flatten on knotting to give knot security superior to any other monofilament.

**Security through reduced risk of suture extrusion and sinus formation**

The specially smooth monofilament structure of PROLENE suture and its ability to tolerate infection extremely well can lessen the risk present with a braided suture in a contaminated wound.

**Indications:** PROLENE Polypropylene suture may be used wherever a non-absorbable surgical suture is recommended.

**Precautions:** Unused PROLENE sutures may not be autoclaved more than three times by the standard autoclaving method without loss of strength. Care should be taken to avoid damaging the surface of the material with surgical instruments.

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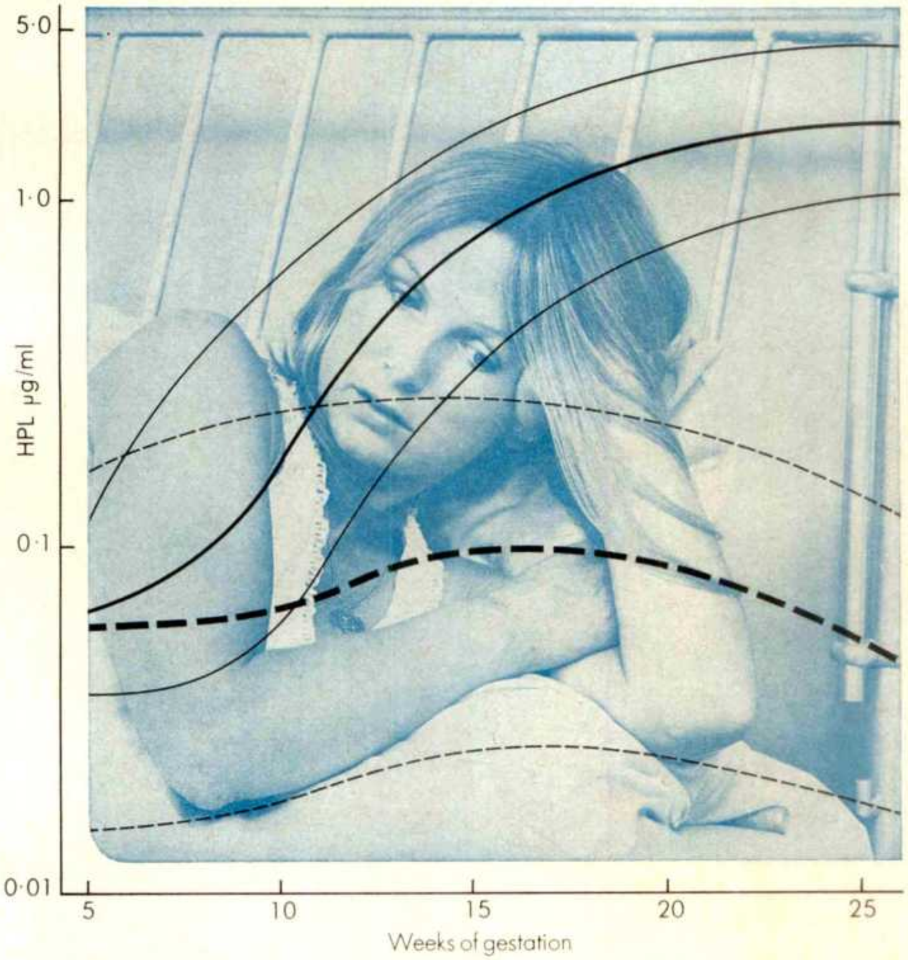

In cases of vaginal bleeding in early pregnancy it is frequently impossible on clinical grounds alone to distinguish between those patients who will abort and those who will proceed to term.

It has been shown that the assay of human placental lactogen (HPL) in maternal serum can often make this distinction.(1) Patients with lower than normal levels usually went on to abort during their first admission, whereas those with normal levels were likely to continue successfully to term. Thus, the HPL assay "can indicate those women in whom abortion is inevitable and could be used to reduce substantially the length of hospital stay in this common complication of early pregnancy."(1)

**Reference** Brit Med J, **3**, 799-801, 1972.

## Human Placental Lactogen

**a rapid, reliable test of placental function**

* no 24-hour collection of urine
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* no risk to either patient or foetus

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2657/8/73

# Menorrhagia
# Metrorrhagia...

# LOW DOSE
# 'Metrulen 50

## *Low total steroid intake

Patients suffering from menorrhagia and metrorrhagia can be subjected to a high daily steroid intake and in some chronic cases this may be continued indefinitely. METRULEN 50 is effective at a daily steroid intake of only 2.05 mg.

## *Low cost

METRULEN 50 represents economy, a days therapy costing less than 4p. Some extensively used preparations can cost over four times as much.

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METRULEN 50 contains ethynodiol diacetate, a progestogen of proven value in the control of menorrhagia.
A little oestrogen has been added for extra cycle control and to minimise breakthrough bleeding. This will mean extra comfort and convenience for your patients.

## *Once-a-day convenience

Unlike high dose progestogen treatments, the recommended dosage of METRULEN 50 is only one tablet a day.

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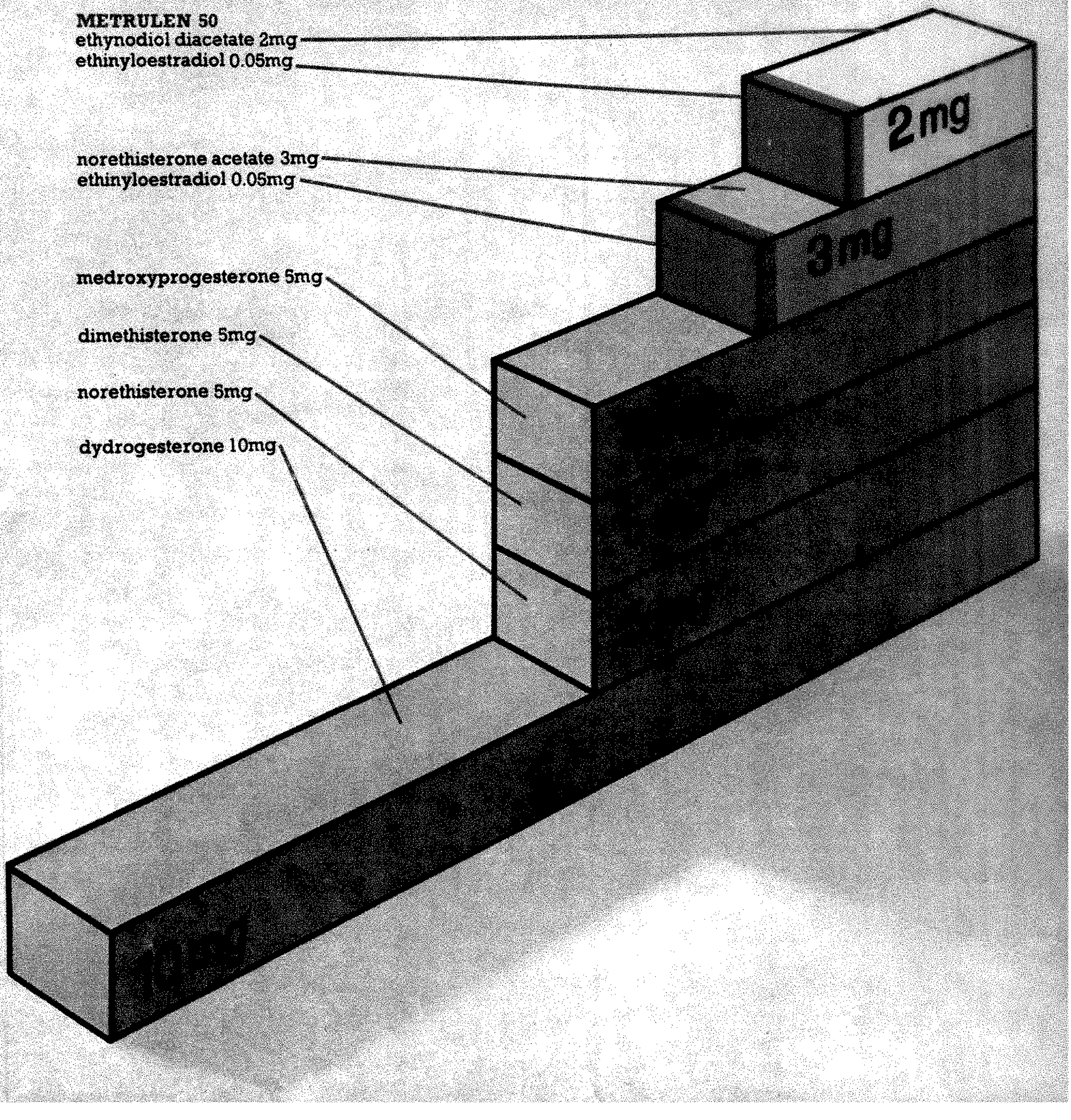
## Steroid content/tablet values of some of the more commonly used preparations for menorrhagia and metrorrhagia



**METRULEN 50**
ethynodiol diacetate 2mg
ethinyloestradiol 0.05mg

norethisterone acetate 3mg
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medroxyprogesterone 5mg

dimethisterone 5mg

norethisterone 5mg

dydrogesterone 10mg

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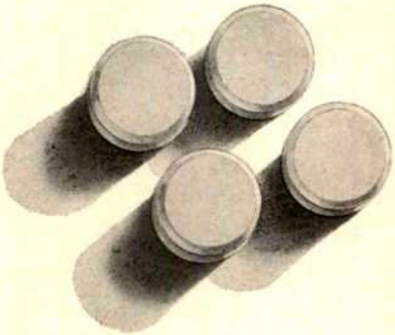

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“Nimorazole was also given orally to 146 women. 88 were given a single dose of 2 g. 58 were treated with their sex partners, each receiving a single 2 g dose. The cure rates were 92 and 96 per cent respectively and there were no serious side-effects.”

*Brit J vener Dis* (1973), **49**, 310

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NEW PRESENTATION

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- Patient has greater freedom of movement.
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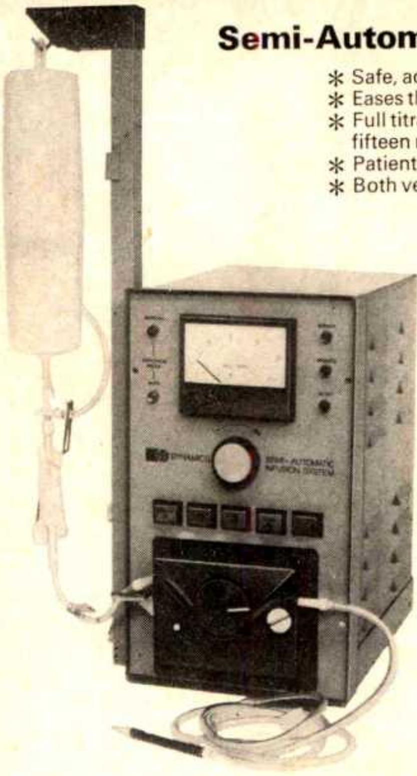

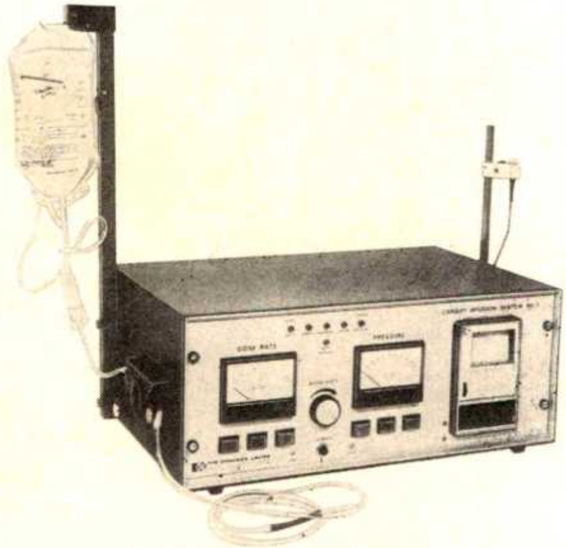

For the inducement of labour, the Cardiff Infusion System provides a continuous and accurately controlled intravenous infusion of oxytocin by peristaltic pump.

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(dinoprostone)

## for Induction of Labour

- **The first new oxytocic agent in nearly 50 years**
- **The first oxytocic that influences uterine activity at any stage of gestation**
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- **For use by intravenous and oral route for induction of labour**

Prostin E2 is Upjohn's synthetic form of prostaglandin E2. This first example from a new world of medicine is specifically indicated for the stimulation of uterine musculature.

Clinical trial work carried out on Prostin E2 in major obstetric centres in the United Kingdom has demonstrated successful induction of labour in 93% of patients by the intravenous route.

Side effects noted have not been severe, and have included nausea, vomiting, diarrhœa and transient reddening of the course of the infused vein.

Prostin E2 offers predictability of action in obstetric use.

Prostin E2 is available for use only in hospitals and clinics with specialised obstetric and gynaecology units.

**Prostin E2** Oral Solution

Supplied as 0.5ml. ampoules containing dinoprostone 10mg/ml. in N.N dimethylacetamide (DMA).

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Supplied as 0.75ml. ampoules containing dinoprostone 1mg/ml. in ethanol (for intravenous use).

*Full product information available on request*

**Prostin E2**

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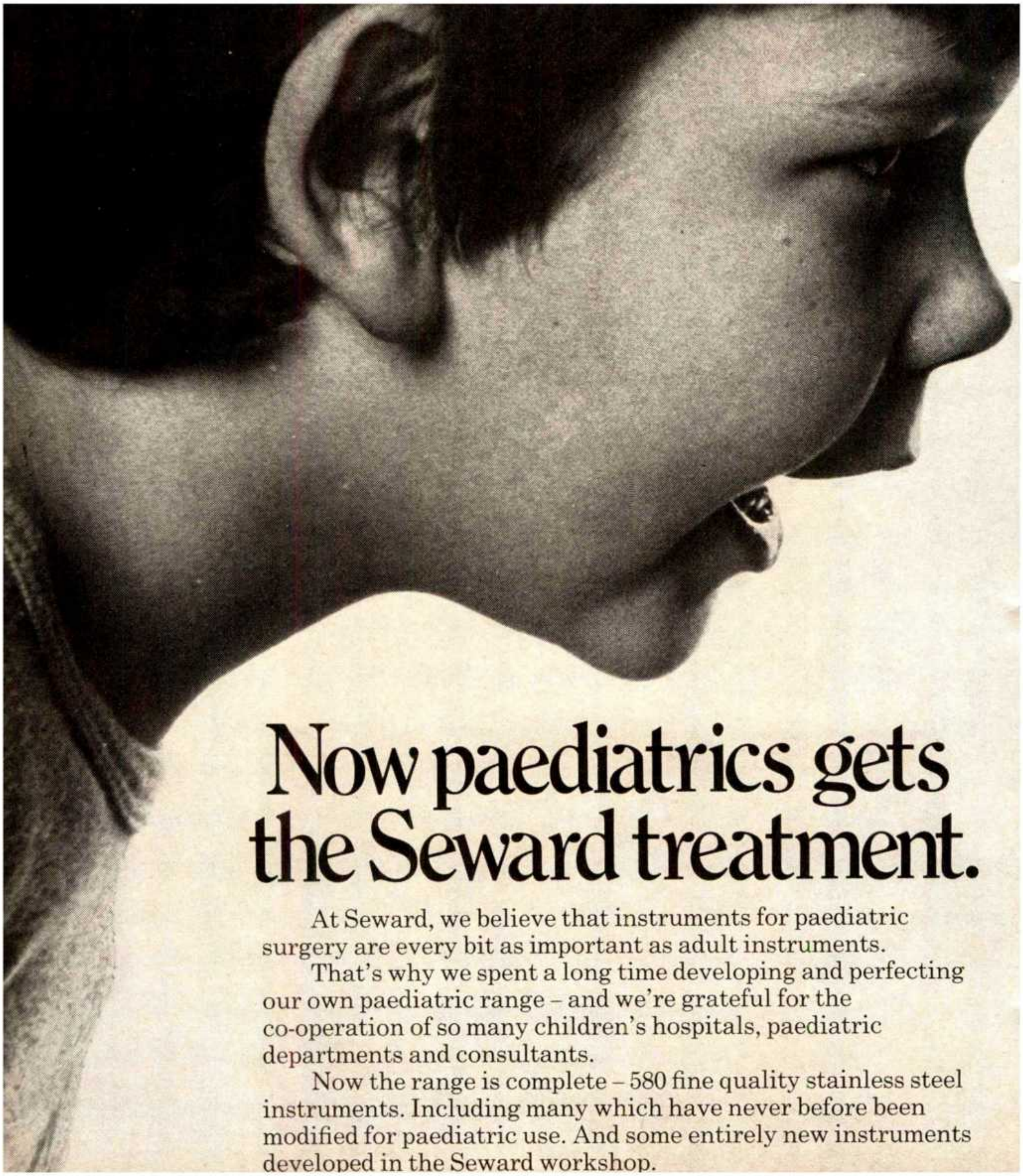

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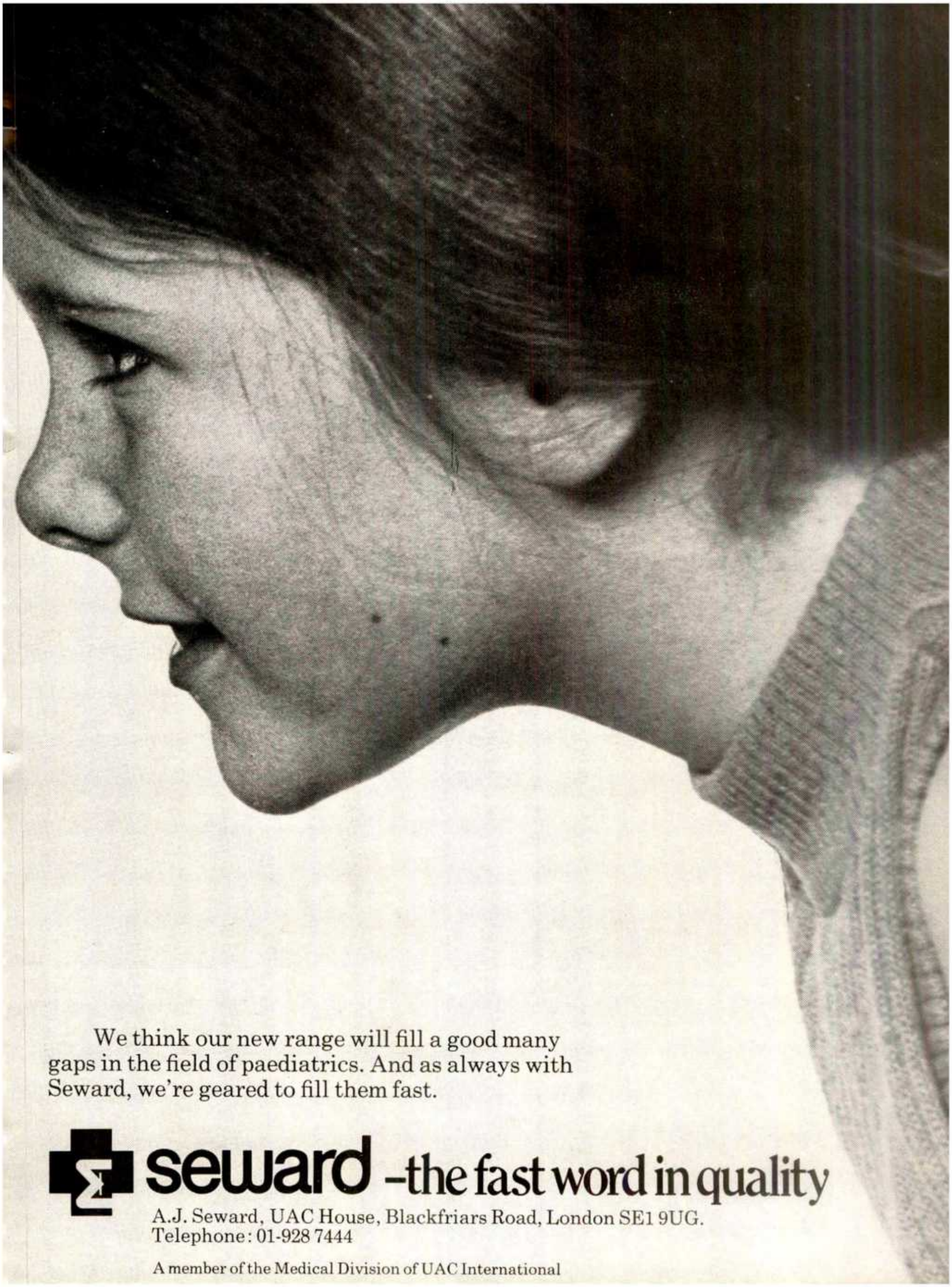

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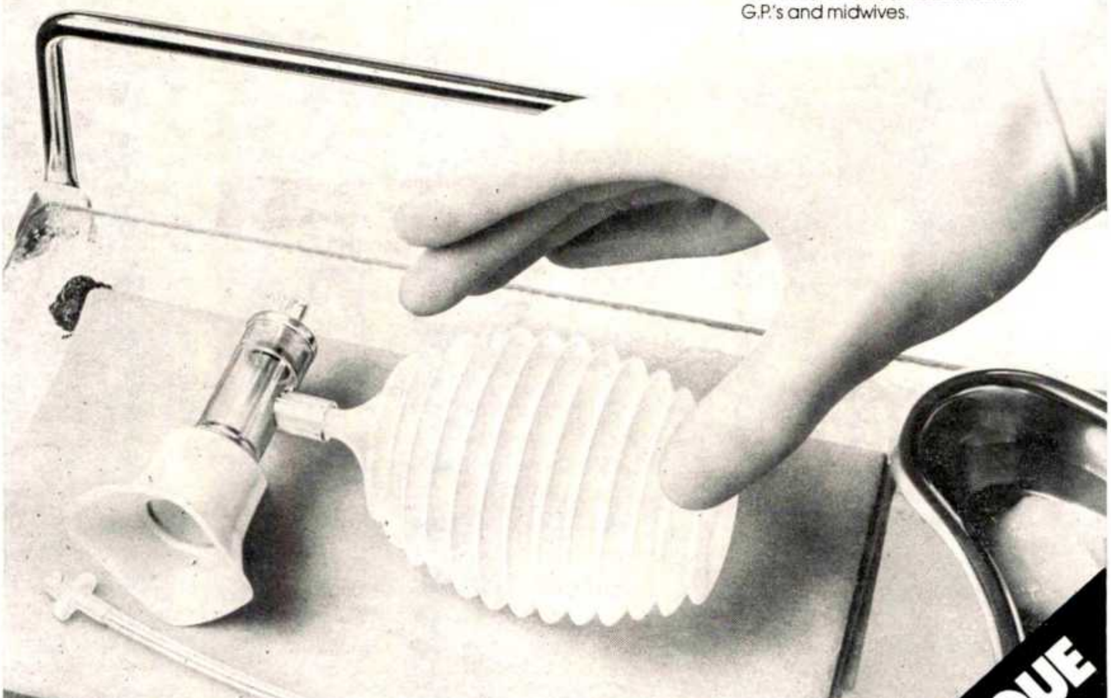

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1 **World Med., 8,** (19), 73, 1973
2 **Gen. Practit.,** p. 19, 13 July, 1973
3 **Practitioner, 212,** 115, 1974
4 **Br.Med.J., iv,** 146, 1971
5 **Ibid., i,** 207, 1971

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British Journal of Obstetrics and Gynaecology

VOL. 82 No. 5 NEW SERIES MAY 1975

# ANTEPARTUM DIAGNOSIS OF THE "TERMINAL" FETAL STATE BY CARDIOTOCOGRAPHY

BY

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## Summary

Since 1969 it has been the policy of this department to perform regular cardiotocograms on all patients admitted during the antenatal period for severe hypertension, with or without proteinuria, and on those in whom poor intrauterine fetal growth was suspected. In five such patients where the fetus died *in utero*, beat-to-beat variation was reduced and late decelerations were observed. In a further twelve patients similar changes made delivery by elective Caesarean section appear advisable. Half of the infants so delivered had severe metabolic acidosis at birth.

CARDIOTOCOGRAPHY has achieved widespread recognition as a method of monitoring the state of the fetus during labour (Caldeyro-Barcia *et al.*, 1968; Hon, 1963). Unfortunately, physiological events occurring during labour can impose changes on the fetal heart rate patterns which make it difficult to diagnose a truly pathological state in the fetus. To avoid these difficulties it has been suggested that cardiotocograms be recorded before the onset of labour (Hammacher *et al.*, 1968; Kubli *et al.*, 1972; Sureau *et al.*, 1970); infants then diagnosed as being "at risk" could be delivered by elective Caesarean section thus avoiding the stress of labour.

In this paper we report our experience with antepartum cardiotocography in the diagnosis of the "terminal" fetal state. By this is meant that the fetus under investigation is so close to dying *in utero* that its condition at the time of making the cardiotocographic recording can reasonably be described as "terminal".

## PATIENTS AND METHODS

During 1969 it was decided that all patients admitted to the wards of this hospital with a diagnosis of hypertension (defined as a persistent diastolic pressure greater than 90 mm. Hg after a period of rest) with or without proteinuria, and those in whom intrauterine growth retardation (IUGR) was suspected would have daily cardiotocograms (CTGs) recorded. Such recordings were made with a Hewlett-Packard cardiotocograph type 8020A, with a phonotransducer, and were of at least 15 minutes duration. The paper speed was 2 cm. per minute. Two groups of patients will be discussed; the first group comprised five patients in whom cardiotocographs were recorded shortly before the infants died *in utero*. A brief account of their past obstetric history, present condition and therapy is given in Table I. The second group comprised 12 patients who were delivered by elective Caesarean section shortly after cardiotocography had shown similar changes which suggested to us that the fetus was at grave risk of dying *in utero*. Their details are given in Table II.

## RESULTS

*Intrauterine death group*. This was a mixed group in that two patients were having their first pregnancy, one had only had a previous therapeutic termination and the remaining two were multigravidae. The linking factor was that all were suspected of having poor intrauterine fetal growth, associated with hypertension or pre-eclampsia in four, and with a chromosomal aberration in one (Table I). Each had had more than one CTG but the features to be described relate to the final recording prior to fetal death (Fig. 1). These were:

1. A base line fetal heart rate in the normal range (120 to 160 beats per minute).
2. Decrease and often disappearance of beat-to-beat variation, the so-called "silent" pattern.
3. "Late" decelerations in relation to the naturally occurring uterine contractions as described by Hon (1963). These decelerations were usually shallow.

Three of the five stillborn infants were of low birthweight for gestation; the placental weights appeared appropriate to birthweight and all placentae were infarcted (Table III).

With the recognition that these cardiotocographic features were of such grave prognostic significance we began to deliver patients with similar recordings by elective Caesarean section.

*Caesarean section group*. Six patients had severe pre-eclampsia and five of the seven multiparous patients had bad past obstetric histories.

At the time of the last CTG recording, one patient was of 37 weeks gestation, four were at 35 weeks gestation and the remainder were of 34 weeks gestation or less, the shortest being 29 weeks gestation. When the CTG features described above were found (Fig. 2), the patients were usually delivered by Caesarean section within 10 hours. The fetal outcome and follow-up are summarized in Table IV. Of the nine infants in whom the appropriate blood samples could be obtained at delivery, four had metabolic acidosis. In two others there were indications of

TABLE I

*Intrauterine death group: past obstetric history and diagnosis*

| Patient number | Number of present pregnancy | Obstetric history | Present diagnosis |
|---|---|---|---|
| 1 | 3 | I. IUD 34 w. 1950 g.<br>II. early abortion | hypertension<br>IUGR |
| 2 | 2 | I. therapeutic abortion | hypertension<br>IUGR |
| 3 | 5 | I. neonatal death (multiple congenital anomalies)<br>II, III and IV: early abortions | IUGR, chromosomal aberration |
| 4 | 1 | – | eclampsia IUGR |
| 5 | 1 | – | pre-eclampsia IUGR |

IUD = intrauterine death; IUGR = intrauterine growth retardation

TABLE II

*Caesarean section group: past obstetric history and diagnosis*

| Patient No. | Number of present pregnancy | Obstetric history | Present diagnosis |
|---|---|---|---|
| 1 | 1 | – | eclampsia |
| 2 | 3 | Two normal pregnancies | superimposed toxaemia and IUGR |
| 3 | 4 | Three early abortions | IUGR |
| 4 | 5 | I: premature delivery (neonatal death)<br>II. premature delivery<br>III. early abortion<br>IV: IUD 30 weeks, 940 g. | IUGR |
| 5 | 1 | – | hypertension IUGR |
| 6 | 4 | I. IUD 28 weeks<br>II. IUD 26 weeks (700 g. placental infarct)<br>III. early abortion | IUGR |
| 7 | 5 | normal | IUGR |
| 8 | 1 | – | severe pre-eclampsia + IUGR |
| 9 | 1 | – | severe pre-eclampsia + IUGR |
| 10 | 5 | I, II: term deliveries; infant weight about 2 kg.<br>III and IV: early abortion | severe pre-eclampsia |
| 11 | 1 | – | IUGR |
| 12 | 5 | I: normal pregnancy<br>II to IV: abortions | superimposed severe pre-eclampsia |

IUGR = intrauterine growth retardation; IUD = intrauterine death)

TABLE III

*Intrauterine death group: outcome of pregnancy. Birthweight and placental weight centiles were calculated from the Amsterdam growth curves (Kloosterman, 1970)*

| Patient No. | Maturity at fetal death (weeks) | Birthweight | | Placenta | | |
|---|---|---|---|---|---|---|
| | | g. | centile | weight (g.) | weight centile | Description |
| 1 | 32 | 1410 | 18 | 230 | 9 | 50% infarction and thrombosis |
| 2 | 33 | 1000 | <2·3 | 215 | 6 | 20% infarcted |
| 3 | 37 | 1200 | <2·3 | 175 | <2·3 | 5% infarcted |
| 4 | 33 | 1380 | 13 | 330 | 38 | partially abrupted, 60% infarction and thrombosis |
| 5 | 34 | 970 | <2·3 | 125 | <2·3 | several infarcts |

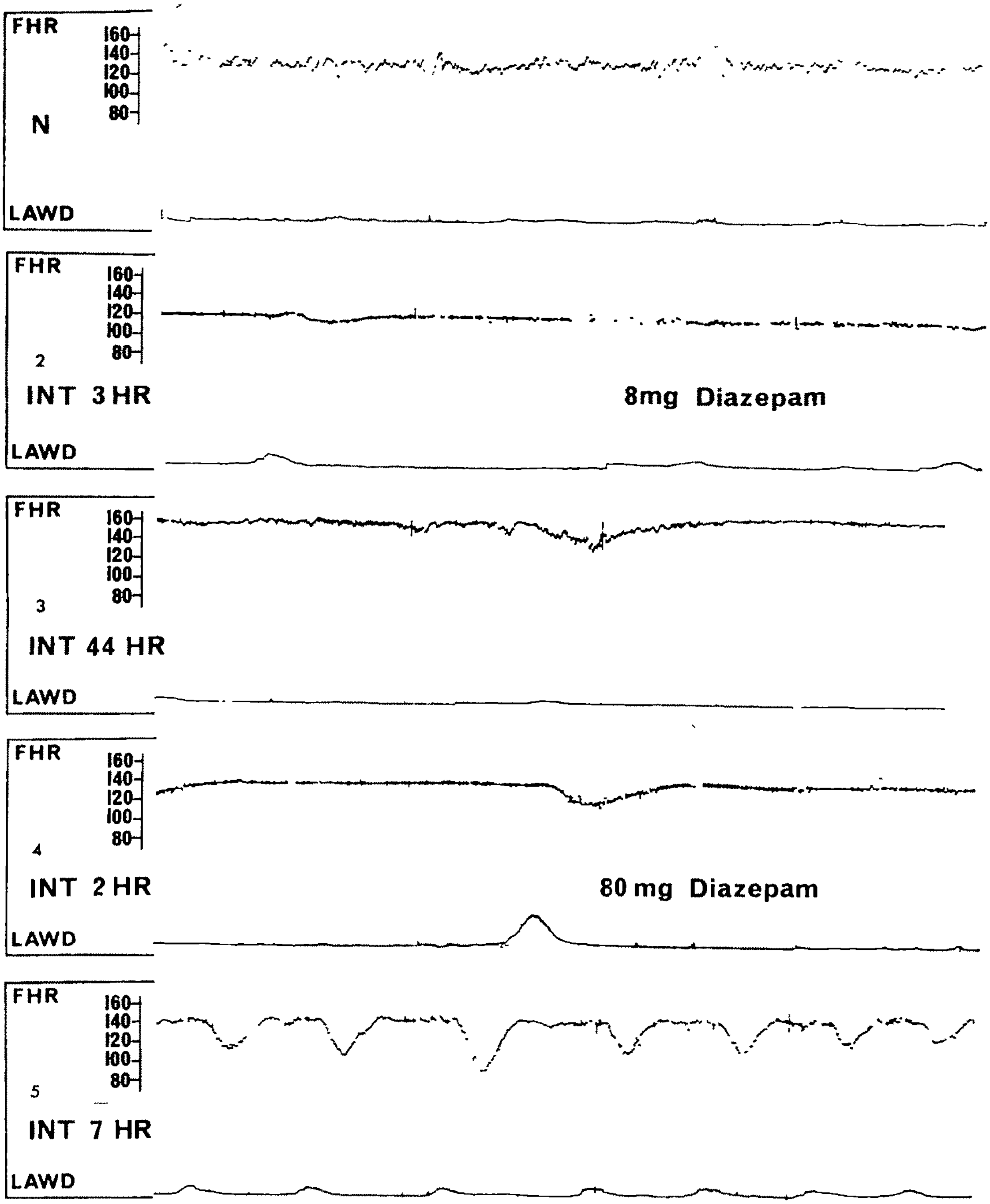

FIG. 1

Normal and "terminal" CTG's. N is a normal CTG in a patient on no medication and 2 to 5 are "terminal" CTG's, the numbers corresponding to those in the first column of Tables I and III. Each recording lasts 20 to 25 minutes. Relevant medication is indicated in daily doses. (FHR = fetal heart rate; LAWD = local abdominal wall displacement; INT = interval between CTG and fetal death.)

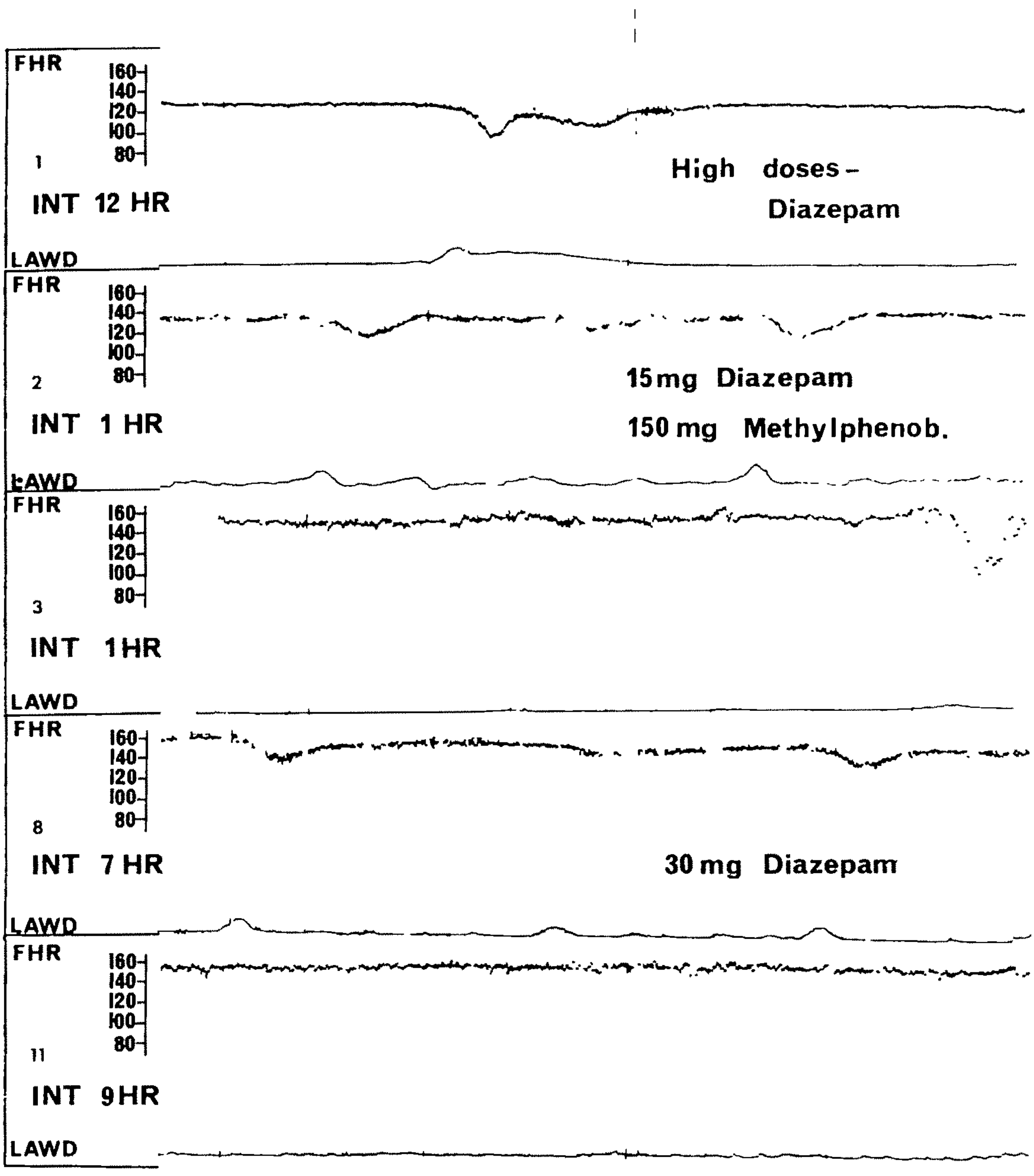

FIG. 2

Five of the twelve CTG's preceding Caesarean section. Numbers correspond with those given in the first column of Tables II and IV. Relevant medication is indicated in daily doses. (FHR = fetal heart rate; LAWD = local abdominal wall displacement; INT = interval between CTG and Caesarean section.)

severe fetal jeopardy. The clinical impression of poor intrauterine growth was confirmed after birth: 10 of the 12 infants were below the 10th centile (five being below the 2·3rd centile) and the remaining two were below the 25th centile. One infant suffered severe brain damage and three died in the neonatal period. The causes of neonatal death were sepsis at four days of age (Patient 5), hyaline membrane disease during the first day (Patient 6) and ventricular haemorrhage at two days (Patient 8).

All placentae were severely pathological, either too small for gestational age or infarcted, or both. In one a retroplacental clot was found.

## DISCUSSION

The most striking feature was that all 17 placentae showed infarcts, thrombosis or retroplacental clots, indicating that the capacity of the fetal "supply-line" was severely reduced. Whatever the primary cause of the placental pathology may have been, there can be little doubt that this ultimately led to fetal death *in utero* or metabolic acidosis of the newborn infants delivered by elective Caesarean section. As the interval between induction of anaesthesia

TABLE IV

*Caesarean section group: outcome of pregnancy and follow-up. Birthweight and placental weight centiles were calculated from the Amsterdam growth curves (Kloosterman, 1970)*

| Patient No. | Maturity at Caesarean section | Birthweight | | Placenta | | | Feto-maternal base deficit difference (mEq./l.)* | | Notes and follow-up |
|---|---|---|---|---|---|---|---|---|---|
| | (weeks) | g. | centile | weight g. | weight centile | description | umb. artery blood | umb. vein blood | |
| 1 | 32 | 1590 | 23 | 270 | 17 | partial abruptio | | 12 | severe brain damage |
| 2 | 35 | 1900 | 9 | 290 | 9 | 10% infarction | 16 | | baby normal at 15 months |
| 3 | 34 | 1340 | 7 | 200 | 3 | | 4·5 | | baby normal at 15 months |
| 4 | 32 | 860 | <2·3 | 210 | 6 | 70% infarction | | 2 | baby normal at 10 months |
| 5 | 32 | 840 | <2·3 | 150 | 3 | | | 1·5 | neonatal death |
| 6 | 29 | 780 | <2·3 | 130 | 3 | multiple infarcts | | | base deficit of umbilical art. blood was 10 mEq./l. Neonatal death |
| 7 | 35 | 1585 | 3 | 180 | <2·3 | 20% infarction | | 0 | baby normal at 15 months |
| 8 | 34 | 1475 | 10 | 250 | 8 | 50% infarction | 5 | | neonatal death |
| 9 | 35 | 1470 | <2·3 | 225 | 4 | several infarcts | | | Apgar score 3 at 3 min., baby normal at 26 months |
| 10 | 34 | 1650 | 8 | 200 | 3 | | 0 | | baby normal at 16 months |
| 11 | 37 | 1670 | <2·3 | 255 | 3 | | | | base deficit of fetal capillary blood 4 mEq./l. at 1 hour, baby normal at 18 months |
| 12 | 35 | 2220 | 22 | 420 | 38 | 10% infarction | −5 | | baby normal at 20 months |

* $\Delta$ BD$_{\mathrm{Hb5}}$ (Jacobson, 1970)

and delivery by Caesarean section was never more than twelve minutes, with a mean of seven minutes, the degree of metabolic acidosis found in the newborn seems likely to reflect the situation *in utero*.

Our findings appear to confirm those in the literature, at least as far as patients with hypertension and intrauterine fetal growth retardation are concerned. Nevertheless, a few questions remain to be answered. First, neither our data nor those in the literature prove beyond doubt that a fetus cannot survive for a considerable time after the CTG has become "terminal". On the other hand, a fetus might die without first having shown "terminal" signs in its CTG.

The first possibility is unlikely, as in none of the five cases of intra-uterine death had a silent pattern and late decelerations been present for longer than a few days. However, not all fetuses in the Caesarean section group were severely acidotic, so that the precise time relationship remains problematic. As for the second possibility, we did not see one CTG-monitored case of intrauterine death from the same causes in the entire period of the study with a normal CTG shortly before death.

It can be questioned whether the characteristic appearance of the "terminal" CTG is induced by influences other than the fetal condition. The effect of drugs, especially sedatives, is well known and was, in fact, recognizable in those of our patients who received them. This does not alter the fact that in all patients not receiving a sedative beat-to-beat variation was reduced in comparison with normal and that late decelerations are not known to be caused by medication. Another factor influencing irregularity, tachycardia, was not present in any of the recordings. It must therefore be concluded that fetal condition is primarily responsible for the "terminal" aspect of the CTG, but that the silent pattern can be aggravated by the use of sedatives.

Further studies should compare series of CTGs in pregnant women with hypertension and poor intrauterine growth in whom the state of the fetus becomes "terminal" with such series in women whose fetus remains in a relatively good condition. To make comparison possible some form of quantitation must be sought. Much work in the field of data processing must be done to quantify less obvious changes in the CTG in order to make identification of preterminal changes possible. If the tentative conclusion this study leads to, that no fetus dies *in utero* from chronic or subacute "placental insufficiency" without having shown clearly identifiable changes in its CTG can be confirmed by such longitudinal studies, then antepartum cardiotocography might become the most important source of information about the immediate state of the fetus.

## ACKNOWLEDGEMENT

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# EPIDURAL ANALGESIA, FETAL MONITORING AND THE CONDITION OF THE BABY AT BIRTH WITH BREECH PRESENTATION

BY

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## Summary

Between December 1970 and March 1973, 138 patients with a singleton fetus presenting by the breech after 36 weeks of pregnancy were deemed suitable for vaginal delivery under epidural analgesia; 130 were delivered vaginally, 10 of them by breech extraction. There was one stillbirth and no neonatal deaths. Epidural analgesia for vaginal breech delivery seemed beneficial. In 65 cases it was possible to compare the umbilical vein pH with the Apgar score at one minute. In 35 patients a continuous recording of the fetal heart rate was used to predict the Apgar score at one minute and the results are discussed.

IN the management of breech presentation, opinions range from those who advocate a high Caesarean section rate (Patterson *et al.*, 1967; Wright, 1959) to those who recommend elective breech extraction under general anaesthesia at full dilatation of the cervix (Dodek *et al.*, 1970). Although conduction analgesia has been used for many years in obstetric practice there remains a concensus of obstetric and anaesthetic opinion that such analgesia may be inappropriate for vaginal breech delivery (Moir, 1971; Crawford, 1972). This is based on the premise that the incidence of breech extraction will be increased as a result of a reduction of the maternal expulsive efforts with consequent increase in fetal birth injuries (Tank *et al.*, 1971).

The particular problems encountered during delivery of the aftercoming head have been summarized by Eliot and Hill (1972). In addition to the fetal risks of intracranial haemorrhage from a precipitate or traumatic delivery associated with incomplete dilatation of the cervix, there are the maternal hazards of emergency general anaesthesia. These hazards are potentially avoidable when conduction analgesia is employed.

The principal benefits conferred by conduction analgesia relate to improved maternal relaxation and reduction of maternal and fetal acidosis (Fisher and Prys-Roberts, 1968; Pearson, 1973). Accurate vaginal examination is possible, and descent of the presenting breech is easily and accurately estimated with no maternal discomfort. Premature expulsive efforts by the mother before full dilatation of the cervix are avoided. Of the disadvantages imputed to epidural analgesia, compression of the inferior vena cava, perhaps the most common problem, is easily avoided by nursing the mother on her side.

Another problem, that of depressed uterine action following epidural analgesia (Tyack *et al.*, 1973), is easily solved by the use of oxytocin. In spite of the alleged inability of the mother to contribute to expulsion of the fetus in the second stage, Bonica (1967) has shown that the intrauterine pressure in the second stage with conduction analgesia is close to that in normal labour providing that the mother is actively encouraged to bear down with each contraction. With these considerations in mind, the following study of breech delivery under conduction analgesia was conducted.

## METHODS

At the Jessop Hospital for Women between December 1970 and March 1973, 6355 patients were delivered, with a 5 per cent incidence of breech presentation at delivery. It is the practice in the antenatal clinic to carry out external version at or about the 34th week in both primigravidae and multigravidae. If the breech presentation persists or recurs, then clinical and radiological assessment of the fetus and the maternal pelvis is performed between 36 and 38 weeks.

During the period of study it was our policy to conduct all breech deliveries after 36 weeks gestation, where possible, under epidural analgesia, providing that the forementioned assessments suggested that safe vaginal delivery was practicable. Spontaneous labour was preferred and induction was performed only for some other obstetric indication.

Once labour was established, a careful pelvic examination was performed and continuous fetal monitoring was instituted whenever possible using an electrode attached to the fetal buttock or foot. Uterine activity was monitored by either external or intrauterine tocography. In 35 patients there was available a continuous recording of the fetal heart rate for the 60 minutes preceding delivery. These records were analysed for their "dip area" as described by Tipton and Shelley, (1971).

Epidural analgesia was established either by the lumbar route or through the caudal hiatus, usually by one of the anaesthetic staff. The anaesthetic agent used was bupivicaine (0·25 or 0·5 per cent) without added adrenaline. The policy was to maintain perineal analgesia throughout the labour. Lumbar epidural anaesthesia was employed in 128 of the 138 patients and caudal analgesia in 10 patients. The range of fetal maturity was 36 to 42 weeks (mean 39).

The progress of labour was assessed by vaginal examination at four-hourly intervals during the first stage of labour and maternal effort was not permitted until it was certain that the cervix was fully dilated and the buttocks visible. At the end of the first stage of labour, more frequent examinations were often necessary to determine the rate of advance of the breech.

The technique of breech delivery varied with accoucheur but in the majority of cases involved early disengagement of the legs, the Løvset manoeuvre to release the arms, and application of forceps to the aftercoming head.

The fetal condition at delivery was assessed independently by the paediatrician and obstetrician present by estimation of the Apgar score at one minute. In 65 cases the umbilical vein pH (UvpH) was also measured at birth in blood obtained immediately after division of the cord.

## RESULTS

There were 317 patients with a singleton fetus presenting by the breech after 36 weeks of pregnancy; in 62 of them (20 per cent) elective Caesarean section was performed. Epidural analgesia was employed in 138 patients (44 per cent) and in only eight was delivery eventually effected by Caesarean section, as detailed below. The remaining 117 were delivered vaginally using conventional analgesia, including general anaesthesia in some patients, but this group included many premature and abnormal babies and is therefore not comparable. Nevertheless, in this "non-epidural" group there were 13 deliveries in which difficulty was encountered during delivery of the aftercoming head due to incomplete dilatation of the cervix or precipitate delivery due to lack of maternal co-operation.

### *Labour*

The onset of labour was spontaneous in 95 patients (54 primigravidae, 41 multigravidae) and induced in 35 (20 primigravidae, 15 multigravidae). The lengths of labour were significantly different with respect to parity although the fetal weights and Apgar scores were similar (Table I).

During labour oxytocin was used in 80 patients; in 34 to induce and in 46 to augment labour, usually after institution of the epidural analgesia. The use of oxytocin was not detrimental to the fetus, as assessed by the incidence of fetal distress and by the Apgar scores, neither was there an increased rate of breech extraction in this group.

In this series 80 per cent of birthweights were in the optimum weight range of 2500 to 3750 g.,

TABLE I

*Length of labour, birthweight and Apgar scores*

| | | Primi-gravidae (74 patients) | Multi-gravidae (56 patients) |
|---|---|---|---|
| Length of 1st stage (hours. minutes) | Mean±SD | 9·40±4·85 | 7·52±4·04 |
| | Range | 3·30—26·28 | 2·40—18·10 |
| Length of 2nd stage (minutes) | Mean±SD | 50±33·4 | 28±18·3 |
| | Range | 10—144 | 5—114 |
| Apgar score at one minute | Mean±SD | 7·2±1·6 | 7·3±2·1 |
| | Range | 3—10 | 0—10 |
| Birthweight (g) | Mean±SD | 3124±419·3 | 3226±540·8 |
| | Range | 2200—4080 | 1790—4500 |

7 per cent were less than 2500 and 13 per cent more than 3750 g. Although the majority of large babies were born to multigravidae (see Fig. 1), there was only one case of breech extraction in this group and this was performed for cord prolapse at full dilatation of the cervix.

There were ten cases of breech extraction, five for fetal distress (including one mongol with a short cord) two for failure of progress in the second stage, two for cord prolapse at full dilatation of the cervix, and one for intrapartum fetal death where the fetal heart was found to be absent just before full dilatation of the cervix. In this patient labour had progressed normally but the membranes ruptured very late and no electrode was attached to the fetus. In all other cases maternal co-operation and effort, augmented where necessary by oxytocin stimulation, resulted in controlled assisted breech delivery.

Eight women eventually delivered by Caesarean section; in two the presenting part remained high at full dilatation of the cervix and in each case the baby weighed more than 3750 g. In the other six patients Caesarean section was performed for fetal distress prior to full dilatation of the cervix and in no case was epidural analgesia considered a contributory factor.

*Condition of infant at birth*

Figure 1 shows the relationship between birthweight and Apgar score at one minute.

Figure 2 indicates the relationship between

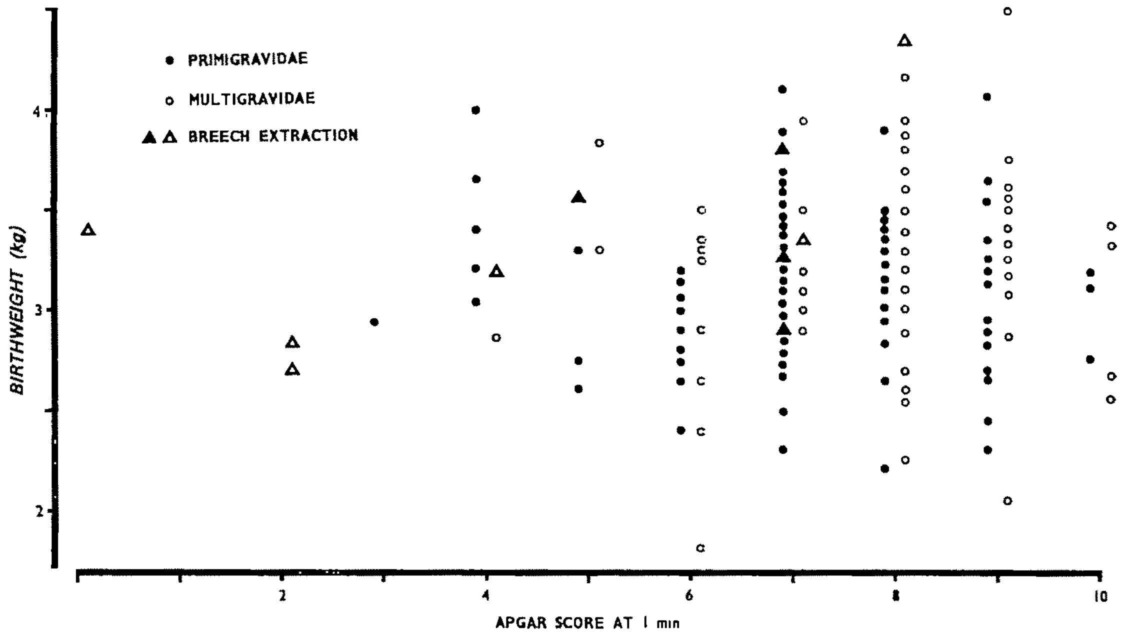

FIG. 1

Relationship between birthweight (kg.) and Apgar score at one minute in 130 vaginal breech deliveries under epidural analgesia.

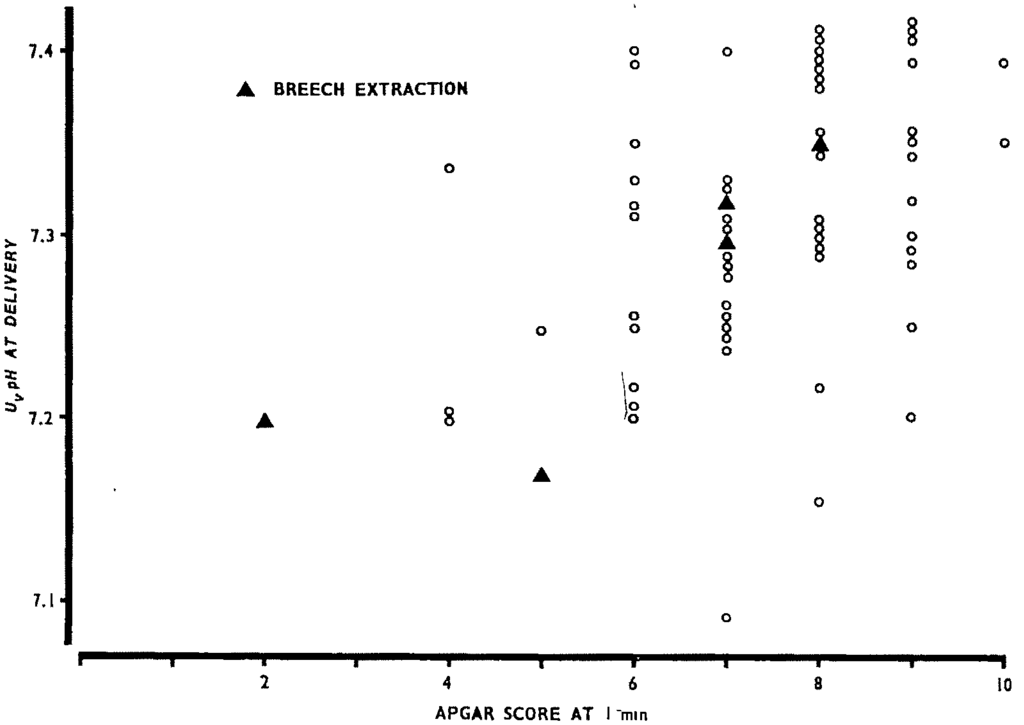

FIG. 2
Relationship between umbilical vein pH at delivery and Apgar score at one minute.

the Apgar score at one minute and the umbilical pH at delivery in the 65 patients in whom a comparison could be made. There were five breech extractions: in two cases for failure to progress in the second stage, in two for fetal distress and in one because of cord prolapse. Three babies had a UvpH below 7·20 and an Apgar score of 5 or more whereas only one had UvpH of 7·20 and an Apgar score of 2.

Figure 3 shows the relationship between UvpH at delivery and the length of the second stage. The latter exceeded one hour in 10 patients, but none of the babies appeared seriously compromised. Whether the fetal condition was assessed by UvpH or Apgar score there was little evidence to suggest that the length of the second stage exerted an important effect on the fetus.

There was a total of 35 patients in whom the fetal heart had been monitored continuously for at least one hour prior to delivery. The relationship between the dip area (Tipton and Shelley, 1971) and UvpH is expressed in Figure 4. The regression line of this relationship is significant ($P<0·01$).

When comparing predicted and actual Apgar scores at one minute (Fig. 5), only three babies fell into the false normal range with a predicted Apgar score which was higher than the actual.

## DISCUSSION

The avoidance of fetal morbidity and mortality during breech delivery must remain the obstetrician's prime concern, and breech extraction, particularly under general anaesthesia, is a major contributory factor to fetal morbidity. With epidural analgesia maternal co-operation ensures not only a low incidence of breech extraction but also the safe and speedy accomplishment of this procedure when indicated. It is noteworthy that 5 out of 17 of those babies delivered with an Apgar score of 5 or less at one minute were delivered by breech extraction for fetal distress. The remaining babies delivered by breech extraction were all in good condition and included two cases in which the second stage of

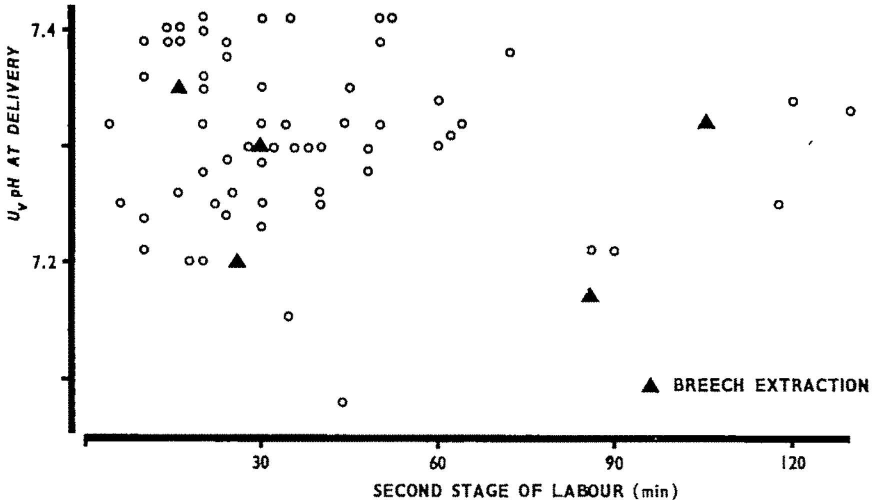

FIG. 3
Relationship between umbilical vein pH at delivery and length of second stage.

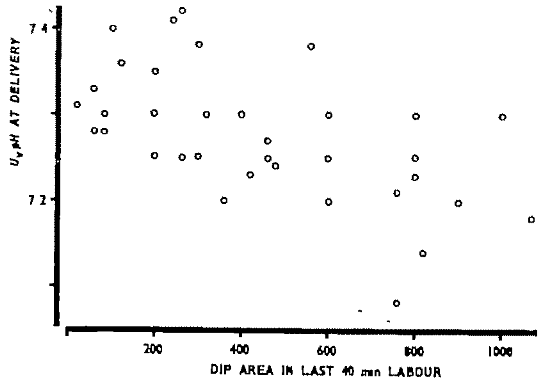

FIG. 4
Relationship between umbilical vein pH at delivery and "dip area" of Tipton and Shelley (1971).

labour had been unduly prolonged. There were no maternal complications attributable to epidural analgesia.

Overall, 87 per cent of the babies had an Apgar score of 6 or more at one minute. There was one stillbirth which might have been avoided if the fetal heart rate had been continuously monitored during labour. The results we obtained in the 35 patients who had been monitored in this way for the 60 minutes preceding delivery showed that the condition of the fetus was fairly accurately reflected by continuous fetal heart rate recordings. This is fortunate, because the appearance of meconium during labour with breech presentation is often due to compression of the fetal abdomen and nothing else. Furthermore, fetal blood sampling when the breech presents may not be as reliable a guide of fetal distress as continuous monitoring in view of the

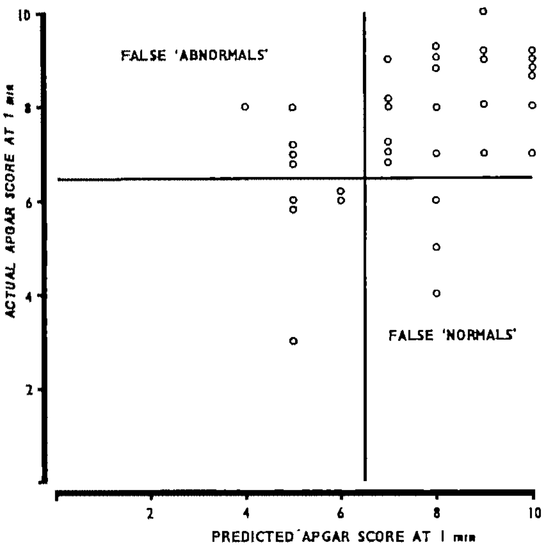

FIG. 5
Correlation of actual and predicted Apgar scores at one minute (Tipton and Shelley, 1971).

decreased oxygenation secondary to increased stasis of blood flow in the breech.

## ACKNOWLEDGEMENTS

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# PREVIOUS REPRODUCTIVE HISTORY IN MOTHERS PRESENTING WITH SPONTANEOUS ABORTIONS

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## Summary

Information on the previous reproductive history of 3467 mothers presenting with a spontaneous abortion is compared with that of a control series presenting with a livebirth. In 1384 abortions the chromosome constitution of the fetal products was determined. The mothers who had delivered a chromosomally normal abortion more often had a history of previous repeated abortions, while those of chromosomally abnormal abortions tended to have fewer repeated abortions but an excess of previous viable children with Down's syndrome. Data are also presented on 23 mothers with two spontaneous abortions of known karyotype; these, together with similar data obtained elsewhere, made it possible to estimate an overall rate of recurrence of chromosome defects in conceptions of the same parents, and the probability that affected fetuses will survive to a viable stage in pregnancy. The clinical and genetic implications of the findings are briefly discussed.

IT has been variously estimated that between 15 and 25 per cent of all conceptions end as spontaneous abortions, and a high proportion of the latter are chromosomally or otherwise abnormal. There is a tendency for abortions to recur in subsequent pregnancies and Rowley *et al.* (1963) and Warburton and Fraser (1964) showed that the recurrence was often at the same stage in pregnancy, However, only in a relatively small proportion do we know the cause of the recurrence and whether concordant anomalies recur, and there is little information on the risk of abnormality in surviving progeny of the same parents.

The study of two series of spontaneous abortions has provided an opportunity for further enquiry into these particular questions.

## METHOD

With the co-operation of the medical and nursing staff of gynaecological units in and around London, we interviewed mothers who had had a spontaneous abortion between 1968 and 1974. Wherever possible fetal products of conception were collected and cultured for chromosome analysis, and a macroscopic examination was carried out.

The study of the first 847 mothers of abortions and the same number of age- and race-matched control mothers of normal babies was planned to enquire into the effects of preconceptional

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TABLE I

*Number of mothers interviewed by age-group*

| Maternal age | Abortions | | | | Control | |
|---|---|---|---|---|---|---|
| | Series II (1971–1974) | | Series I (1968–1971) | | Series I (1968–1971) | |
| | No. | Per cent | No. | Per cent | No. | Per cent |
| <20 | 224 | 8·5 | 51 | 6·0 | 44 | 5·2 |
| 20–29 | 1527 | 58·3 | 494 | 58·3 | 482 | 56·9 |
| 30–39 | 748 | 28·5 | 264 | 31·2 | 285 | 33·7 |
| 40+ | 117 | 4·5 | 38 | 4·5 | 36 | 4·2* |
| Not known | 4 | 0·2 | – | – | – | – |
| Total | 2620 | 100·0 | 847 | 100·0 | 847 | 100·0 |

* This proportion in all livebirths in Greater London in 1969 was 2·1 per cent (Registrar General, 1971).

radiation (Alberman *et al.*, 1972). The control patients were delivered in the same hospital as those with abortions, at about the time that the patients who had abortions would have been due. The second series, comprising 2620 mothers of spontaneous abortions, was designed to study the effects of previous oral contraception, and no controls were seen. The two abortion series were similar in maternal age distribution (Table I), and were largely obtained from the same hospitals, the second series following directly upon the first. The controls for the first series are therefore also comparable with the second series, although the data are presented separately. Mothers of twin abortions were counted only once in the study, but the 86 mothers who entered the study more than once, were counted as new cases each time they aborted. The number of specimens received and their chromosome constitution are given in Table II.

TABLE II

*Specimens received and their karyotype*

| Karyotype | Series II | Series I | Total |
|---|---|---|---|
| Unknown | 1644 | 439 | 2083 |
| Normal | 687 | 305 | 992 |
| Abnormal | 289 | 103 | 392 |
| trisomy | 143 | 43 | 186 |
| 45, X | 68 | 28 | 96 |
| other | 78 | 32 | 110 |
| All | 2620 | 847 | 3467* |

* The 56 pairs of twins counted once only and categorized as follows:

Normal—27 pairs, both normal; 13 pairs, one normal and one unknown.

Abnormal—1 pair, one abnormal and one unknown.

Unknown—15 pairs, both unknown.

We obtained from the mothers details of all previous pregnancies including information about any defects in previous viable births. This information was checked from the hospital records.

## FINDINGS

### *Outcome of previous pregnancies*

Table III shows the overall success rate of previous pregnancies of mothers who presented with a spontaneous abortion. The general picture is best seen by comparing the last two columns, Series I abortions and Series I controls, for these were mothers matched for age and for race. The mothers of the abortions were less often primigravidae than those of the controls, and those who had been pregnant before had, on average, had slightly more pregnancies, 2·6 each compared with 2·4. This was probably because many more of the previous pregnancies of the abortion series had ended in abortion. The abortions in excess were largely spontaneous but there was also a small excess of induced abortions. Thus, although the mothers of the abortions had become pregnant more often than their controls, they were less successful in keeping pregnancies to a viable stage.

We had collected no controls for Series II, but comparing this with the abortions in Series I, the proportion of previous pregnancies which had ended in spontaneous abortion is the same.

TABLE III

*Success rate of previous pregnancies*

| | Abortions | | | | Controls | |
|---|---|---|---|---|---|---|
| | Series II<br>No. | | Series I<br>No. | | Series I<br>No. | |
| Mothers at risk | 2620* | | 847 | | 847 | |
| Primigravidae | 731 | | 195 | | 278 | |
| | No. | Per cent | No. | Per cent | No. | Per cent |
| Previous pregnancies: | | | | | | |
| Abortion—spontaneous | 1302 | 29·7 | 505 | 30·3 | 256 | 19·0 |
| Abortion—induced | 213 | 4·8 | 42 | 2·5 | 17 | 1·3 |
| Births | 2874 | 65·5 | 1118 | 67·2 | 1072 | 79·7 |
| Total pregnancies | 4389 | 100·0 | 1665 | 100·0 | 1345 | 100·0 |

* Includes 23 with an unknown obstetric history.

There were two main differences between the first and the second series. The mothers of the second series, in keeping with secular change, had, on average, less pregnancies, and they had shared in the general experience of having more induced abortions.

The excess of previous pregnancy losses in the abortion series was accounted for both by mothers who had had only one previous abortion, and by those with a history of repeated losses (Table IV).

Table V shows the outcome of previous pregnancies related to the chromosome constitution of the presenting abortion. The proportion of previous pregnancies ending in spontaneous abortion was much the same in each of the groups. This was surprising in view of the considerably lower maternal age of the chromosomally "normal" group, in which only 2 per cent of the mothers were aged 40 or more, compared with 12 per cent of the mothers of the trisomic abortions. The proportion of previous pregnancies which had been induced abortions was twice as high in the chromosomally "normal" as in the "abnormal" group.

In order to look further into the possibility that, allowing for maternal age, mothers of the "normals" were unduly likely to have previous abortions compared with those of the "abnormals" we compared the reproductive history of mothers in Series I and II with that of their age- and race-matched controls. The largest excess of previous abortions was indeed in the chromosomally "normal" group, in which the mothers of the abortions were 48 per cent more likely than their controls to have had any previous abortion, and 244 per cent more likely to have had three or more. The corresponding figures for the abnormal group were 23 per cent and 45 per cent (Table VI).

In case the inclusion of the 86 patients with repeated abortions who entered the study more than once had biased these findings, the calculations were repeated, including only their first entry, but the results remained very similar.

TABLE IV

*Percentage incidence of previous history of abortion (spontaneous or induced) in multigravidae in the three groups studied*

| No. of previous abortions | Series II | Series I | Controls |
|---|---|---|---|
| 0 | 49·9 | 47·7 | 64·0 |
| 1 | 32·9 | 34·4 | 27·9 |
| 2 | 9·9 | 9·7 | 5·3 |
| 3+ | 7·3 | 8·2 | 2·8 |
| Total | 100·0 | 100·0 | 100·0 |
| No. not known | 23 | – | – |
| Mothers at risk | 1866 | 652 | 569 |

### *Congenital defects in viable siblings*

Table VII shows the overall distribution of defects reported in older siblings by chromosome

TABLE V

*Per cent success rate of previous pregnancies by karyotype of presenting abortion (Series I and II)*

| Outcome of previous pregnancy | Karyotype of presenting abortion | | | |
|---|---|---|---|---|
| | Abnormal | | Unknown | Normal |
| | Trisomy | Other | | |
| Abortion—spontaneous | 30·2 | 28·3 | 28·6 | 32·8 |
| Abortion—induced | 2·2 | 1·8 | 3·9 | 5·7 |
| Births | 67·6 | 69·9 | 67·5 | 61·5 |
| Total | 100·0 | 100·0 | 100·0 | 100·0 |
| Number of pregnancies at risk | 361 | 332 | 3667 | 1694 |

TABLE VI

*Percentage incidence of history of abortion (spontaneous or induced) in multigravidae grouped according to karyotype of presenting abortion and in controls matched for age and race*

| No. of previous abortions | Abortion of abnormal karyotype | | Controls | Abortion of unknown karyotype | | Controls | Abortion of normal karyotype | | Controls |
|---|---|---|---|---|---|---|---|---|---|
| | Series II | Series I | Series I | Series II | Series I | Series I | Series II | Series I | Series I |
| 0 | 52·2 | 48·9 | 58·3 | 51·4 | 49·7 | 66·3 | 45·4 | 44·4 | 62·5 |
| 1 | 35·2 | 33·3 | 30·6 | 31·8 | 33·7 | 27·6 | 34·6 | 35·8 | 27·5 |
| 2 | 6·3 | 7·8 | 4·2 | 10·0 | 9·1 | 4·0 | 11·1 | 11·2 | 7·5 |
| 3+ | 6·3 | 10·0 | 6·9 | 6·8 | 7·5 | 2·1 | 8·9 | 8·6 | 2·5 |
| Total | 100·0 | 100·0 | 100·0 | 100·0 | 100·0 | 100·0 | 100·0 | 100·0 | 100·0 |
| No of mothers at risk | 205 | 90 | 72 | 1176 | 330 | 297 | 485 | 232 | 200 |

TABLE VII

*Defects in previous viable siblings by karyotype of presenting abortion of patients in Series I and II*

| Number of siblings | Karyotype of presenting abortion | | | | Controls Series I |
|---|---|---|---|---|---|
| | Abnormal | | Not known | Normal | |
| | Trisomy | Other | | | |
| At risk | 244 | 232 | 2474 | 1042 | 1072 |
| with Down's syndrome | 5 | 0 | 6 | 0 | 0 |
| with neural tube defect | 3 | 1 | 10 | 5 | 3 |
| with congenital heart disease | 1 | 0 | 2 | 4 | 6 |
| with hare lip and/or cleft palate | 1 | 0 | 2 | 2 | 0 |

constitution of the presenting abortion. The outstanding finding is that amongst the viable siblings of trisomic abortions, 1 in 49 had Down's syndrome, compared with 1 in 412 in the "unknown" group, none in 1042 in the "normal" group and none in 1072 in the controls. This excess of Down's syndrome in the siblings of the trisomies might be easily explained if the presenting abnormality in the abortion had always been trisomy 21. However, this could

Table VIII

*Maternal age and karyotype in chromosomally abnormal and unknown abortions with a sibling with Down's syndrome*

| Presenting abnormality | Maternal age at birth of Down's syndrome child | Maternal age at presenting abortion |
|---|---|---|
| Trisomy G | 39 | 42 |
| Trisomy E | 28 | 31 |
| Trisomy C | 29 | 37 |
| Trisomy D | 38 | 44 |
| Trisomy D | 42 | 44 |
| Not known | 29 | 34 |
| Not known | 33 | 41 |
| Not known | 29 | 34 |
| Not known | 27 | 28 |
| Not known | 31 | 33 |
| Not known | 29 | 38 |

Table IX

*Maternal age and karyotype where two abortions from the same mother were studied*

| No. | Maternal age | First abortion | Maternal age | Second abortion |
|---|---|---|---|---|
| 1 | 38 | 46, XY | 39 | 46, XY |
| 2 | 27 | 46, XY | 27 | 69, XXY |
| 3 | 25 | 46, XY | 25 | 46, XX |
| 4 | 21 | 46. XY | 21 | 46, XX |
| 5 | 25 | 47, XY+C | 27 | 46, XY |
| 6 | 26 | 46, XX | 27 | 46, XY |
| 7 | 26 | 46, XY | 29 | 46, XY |
| 8 | 24 | 46, XY | 25 | 92, XXXX |
| 9 | 19 | 46, XX | 20 | 46, XY |
| 10 | 31 | 46, XY | 31 | 46, XX |
| 11 | 19 | 46, XY<br>46,XY−D +t(DqDq) | 20 | 46, XY−D +t(DqDq) |
| 12 | 32 | 47, XX+C | 34 | 47, XY+D |
| 13 | 19 | 45, X | 20 | 46, XX |
| 14 | 27 | 46, XX | 27 | 46, XY |
| 15 | 35 | 46, XY | 36 | 46, XY |
| 16 | 34 | 46, XY | 36 | 46, XY |
| 17 | 26 | 46, XY | 26 | 46, XX |
| 18 | 27 | 46, XX | 29 | 46, XX |
| 19 | 26 | 46, XX | 31 | 46, XY |
| 20 | 28 | 46, XY | 39 | 46, XX |
| 21 | 21 | 46, XX | 22 | 46, XY |
| 22 | 21 | 46, XX | 22 | 46, XY |
| 23 | 21 | 46, XY | 21 | 46, XY |

only have been the case in the first of the five abortions, where the trisomy was of either chromosome 21 or 22 (Table VIII).

In all 1384 karyotyped specimens there had been 186 trisomies. One might, therefore, expect that amongst the 2474 siblings of abortions of unknown karyotype (Table VII), 186/1384 × 2474 were really siblings of trisomic abortions. If the six siblings with Down's syndrome of the "unknown" group had been derived from these, this would have given a prevalence of 1 in 55, very close to that found in the siblings of the known trisomic abortions. In the case of the neural tube defects and hare lip and cleft palate there was also a small but not significant excess of affected cases in the siblings of the trisomic group, but no such excess appeared in the case of congenital heart disease.

### *Mothers who had had more than one abortion in Series I and II*

There were 86 mothers who came into the combined series more than once, 82 twice and 4 three times. However, we were able to karyotype two abortions from the same mother only 23 times (Table IX). There were seventeen pairs in which both abortions were chromosomally normal, two where both were abnormal, and four where one was normal and the other abnormal.

Boué and Boué (1973) have reported on a series of 43 mothers from whom two abortions were karyotyped. Table X presents the combined results from their series and ours.

### *Overall proportion of chromosome anomalies in previous pregnancies*

From the data we have presented, and making use of the additional information from Boué and Boué (1973) (Table X), an estimate can be made of overall chromosome abnormality rate in previous pregnancies of the different groups.

As a baseline we have looked at the estimated risk in our control Series I, using the overall proportion of normal to abnormal abortions found in our combined series. Of the 1345 previous pregnancies in the controls 273 ended in abortion (spontaneous and other), and it is estimated that 77 (28·2 per cent) of the latter were chromosomally abnormal. There were no recognized chromosome anomalies in the 1072 previous viable births, so that the overall incidence of anomalies in the 1345 previous pregnancies was 5·7 per cent.

Similar estimates were carried out for the

TABLE X

*Mothers with two abortions karyotyped—combined results of Boué and Boué (1973) and the present series*

| First abortion | Second abortion | | | | | All |
|---|---|---|---|---|---|---|
| | Normal | Monosomy | Trisomy | Polyploidy | Translocation | |
| Normal | 24 | 1 | 5 | 4 | 0 | 34 |
| Monosomy | 3 | 0 | 1 | 1 | 0 | 5 |
| Trisomy | 3 | 1 | 14 | 1 | 0 | 19 |
| Polyploidy | 1 | 0 | 3 | 1 | 0 | 5 |
| Translocation | 0 | 0 | 0 | 0 | 3 | 3 |
| All | 31 | 2 | 23 | 7 | 3 | 66 |

abortion series, but the number of previous abortions of abnormal karyotype was calculated using data from Table X, which differentiates between the risk of abnormality when the presenting problem is normal and that when it is abnormal. Where the presenting abortion was of "normal" karyotype it was estimated that 7/31 of the previous 652 abortions would have been of abnormal karyotype; no chromosome anomalies were recognized among the 1042 viable births, so that 8·7 per cent of all previous pregnancies were of abnormal karyotype. Similarly, in the group presenting with an abortion of abnormal karyotype, it was estimated that 25/35 of the 217 previous abortions would have been of abnormal karyotype; 5 of the previous 476 viable pregnancies were known to have Down's syndrome, so that at least 23·1 per cent of all previous pregnancies had an anomaly. For the subgroup of trisomic abortions, with a risk of 18/23 for a recurrence of abnormality in a subsequent pregnancy, it was estimated that the overall previous abnormality rate had been 26·7 per cent, nearly five times as high as in the control series.

## DISCUSSION

### *Risk of recurrence of spontaneous abortion*

Although it has long been known that spontaneous abortions tend to recur, it is only in the last few years that recurrence has been studied in the two major aetiological groups—abortions with and without chromosomal anomaly. The first such study was carried out by Boué and Gueguen (1972) on their large series of abortions of known karyotype. The present series presented a similar opportunity.

The series studied by Boué *et al.* (1973) differs from the present in two important respects. Firstly, they studied only abortions with an estimated developmental age of 12 weeks or less, whereas the present account is of any mother admitted to hospital with a spontaneous abortion. Since the incidence of chromosome anomaly is highest in abortions that are developmentally the most immature this has meant that the overall chromosome anomaly rate is very much lower in ours than in the French series, 28·2 per cent compared with 60·4 per cent (Boué *et al.*, 1973). A more important difference is the fact that the French series involved the active co-operation of patients and it may have been somewhat biased in favour of habitual aborters.

Nevertheless it is instructive to compare their results with ours. Boué and Gueguen (1972) found that 41 per cent of previous pregnancies of mothers presenting with a chromosomally normal abortion, and 39 per cent of those presenting with a chromosomally abnormal abortion, had ended in abortion. These figures correspond with 39 per cent and 31 per cent respectively in the present series (combining all abnormals and including induced abortions and terminations).

From the present series there is a suggestion that recurrence, particularly multiple recurrence, is more common in the chromosomally normal group, especially when the mothers were compared with age-matched controls. From the paper by Boué and Gueguen (1972) it appears that the rate of previous pregnancy loss was greater in the "normal" group In the mothers presenting under the age of 30, this was 37 per cent compared with 33 per cent in the corresponding mothers of the abnormals, although there was no difference between the older groups. It seems therefore that recurrence, particularly repeated recurrence, is more often associated with the loss of chromosomally normal fetuses. This suggests that cytogenetic studies of families in whom recurrent abortion occurs are unlikely to be profitable (Wilson, 1969).

It also appears from the present series that previous induced abortion was more common in the normal group. It has long been recognized that these may be followed by abortion or premature labour in a later pregnancy. These findings further support the view that such subsequent complications are due to maternal rather than fetal factors.

Even mothers presenting with a chromosomally abnormal abortion had a considerable excess loss in previous pregnancies in both the present and the French series. This excess could be accounted for by previous chromosomally abnormal conceptions, or by the fact that several different causes of fetal loss exist in these mothers. If the assumptions we have used in calculating overall abnormality rate in previous pregnancies are correct, the chromosome anomalies in previous abortions of mothers presenting with an abortion of abnormal karyotype fully account for their excess. In this group the estimated proportion of previous pregnancies ending in abortions with a normal karyotype is 9 per cent compared with 14 per cent in the controls; while 22 per cent ended as abortions of abnormal karyotype, compared with 6 per cent in the controls.

### *Risk to viable siblings*

More important was our finding that the prevalence of the most common chromosomal anomaly compatible with life, Down's syndrome, was ten times higher than that expected in siblings of mothers presenting with an abortion of abnormal karyotype. Two patients in whom a previous child had Down's syndrome were reported by the French authors (Boué *et al.*, 1973): one had two subsequent abortions with trisomy E; the other had two abortions of apparently normal karyotype. As always, the calculation of the real risk of recurrence is bedevilled by the general steep rise in risk of trisomy with maternal age. However, only three of the six mothers of trisomic abortions and children with Down's syndrome (five from this series, and one from Boué *et al.*, 1973) and one of the six mothers of children with Down's syndrome who presented with an abortion of unknown karyotype, had been over 40 at the time of the birth of the affected fetus, so this factor alone is unlikely to account for the high recurrence rate.

### *Antenatal mortality of chromosomally abnormal fetuses*

It is only in the subgroup presenting with a trisomic abortion that there are enough viable births with chromosome anomalies to estimate their antenatal mortality. In this group however the overall antenatal mortality of affected fetuses was of the order of that described by Polani (1970), namely, 95 per cent. Out of the estimated 97 previous chromosomally abnormal conceptions only five had survived.

### *Type of recurrence*

Boué *et al.* (1973) pointed out that recurrent chromosomal anomalies are not necessarily identical. Most common is the recurrence of two trisomies of different types, but Boué and Boué (1973) found one patient who had an abortion with trisomy E followed by another with monosomy X, two patients with triploidy followed by a trisomy E, and one with monosomy X followed by triploidy. It is not known whether all these abnormalities result from a form of non-disjunction or whether several different mechanisms are involved.

### *Implications*

The more gestationally mature the abortion the less likely does chromosomal abnormality become, particularly if the aborted fetus appears normal. It is also known that chromosomal anomalies become very much more common with advancing maternal age. Thus an older mother with a history of previous early abortions is very likely to be at high risk of chromosomal anomaly in a subsequent pregnancy, and these factors may legitimately influence a decision on possible amniocentesis for antenatal diagnosis. We have felt obliged to offer such a service to obstetricians looking after mothers who have had a previous trisomic abortion. Conversely, in a young mother with a history of mid-trimester abortions the cause is likely to be unrelated to the karyotype of the conceptions, and amniocentesis is probably not justified.

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# THE EFFECT OF PREGNANCY ON THE RENAL HANDLING OF GLUCOSE

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## Summary

Tubular reabsorption of glucose has been measured during glucose infusion in 29 healthy women during and after pregnancy. All the women had normal glucose tolerance to an oral load, and normal glucose excretion when not pregnant, but exhibited a wide range of daily glucose excretion in pregnancy. Throughout pregnancy the renal reabsorption of glucose is less effective than in the non-pregnant state and, in general, the greater the amount of glycosuria which develops in pregnancy, the less effective is the reabsorption during infusion. *Post partum*, women with minor degrees of glycosuria during the preceding pregnancy return to a normal highly efficient reabsorption performance during infusion, but women who exhibit greater degrees of glycosuria have a reduced capacity to reabsorb even though they are no longer glycosuric after the pregnancy. It is concluded that pregnancy imposes some specific change in the glucose reabsorptive capacity of the proximal tubule and that women with more than usual degrees of glycosuria in pregnancy may, in addition, have an element of tubular damage. This is discussed in relation to other renal function changes in pregnancy in an attempt to explain the characteristic intermittency of clinical glycosuria in pregnancy.

HEALTHY pregnant women excrete more than the usual amounts of glucose in urine. The losses may be ten or more times greater than those of non-pregnant women, but the amounts vary widely from day to day and during the course of a day without any obvious relation to meals (Lind and Hytten, 1972). Normal non-pregnant patterns of excretion are re-established within a week after delivery (Davison and Lovedale, 1974), and the evidence points to an intermittent renal tubular failure to reabsorb glucose, specific to pregnancy.

Earlier investigators (Christensen, 1958; Welsh and Sims, 1960) concluded that the maximum tubular capacity to reabsorb glucose, the so-called $\text{Tm}_G$, is unaltered during pregnancy, and that the tendency to excrete glucose is due to a raised glomerular filtration rate (GFR), which so increases the filtered load of glucose that tubular capacity is overwhelmed.

That view was based on methods and concepts used by the classical workers on renal function (Shannon and Fisher, 1938; Goldring *et al.*, 1940; Smith *et al.*, 1943) and it has become clear that both their results and interpretations were misleading. For example, the estimation of glucose in urine by the traditional methods grossly underestimated the amount of glucose excreted, so that the kidney was credited with a far greater reabsorptive capacity than it had

(Davison and Cheyne, 1972 and 1974), and replotting of the earlier data on which the concept of a ceiling of reabsorption, the $\mathrm{Tm}_G$, was based shows no convincing evidence of any such ceiling (Davison and Hytten, 1974). Eggleton and Shuster (1953) and Dempster *et al.* (1956) were also unable to find evidence of a $\mathrm{Tm}_G$ in cats and dogs; instead reabsorption continued to rise, though at a diminishing rate, as the filtered load increased. Furthermore, it is now clear that glucose reabsorption is related to the state of sodium and fluid reabsorption by the proximal tubule and therefore to all the physiological circumstances which influence that (Keys and Swanson, 1971; Schultze and Berger, 1972); it also varies with alterations in glomerular filtration rate (GFR), and GFR is itself altered by high levels of glucose in the blood (Brøchner-Mortensen, 1973; Kurtzman and Pillay, 1973).

Since the development of the only method so far capable of determining glucose accurately in urine, using the enzymes hexokinase and glucose-6-phosphate dehydrogenase (Barthelmai and Czok, 1962; Schmidt, 1963; Lind *et al.*, 1971) there seems to have been no detailed study of tubular reabsorption of glucose in "glycosuric" and "non-glycosuric" pregnant women. Renschler and von Baeyer (1966) reported briefly without data, that 15 pregnant women, 11 of whom were "glycosuric", showed a reduced ability to reabsorb glucose compared to the usual performance of non-pregnant subjects.

Since "glycosuria" is an extremely variable and as yet unexplained phenomenon during pregnancy, it seemed logical to re-examine tubular reabsorption by modern techniques in women whose patterns of glucose excretion throughout pregnancy were known.

### Subjects and Methods

Twenty-nine normal pregnant women agreed to co-operate in the study after detailed explanation; only two were specifically recruited because routine urine analysis at the antenatal clinic had revealed heavy glycosuria on several occasions. All were in good general health, had normal oral glucose tolerance tests at 30 weeks of pregnancy, and had no family history of diabetes; repeated mid-stream specimens of urine were sterile on culture in all cases. Each patient was delivered of a normal child whose weight at birth was above the 25th centile for gestation as judged by the sex and parity specific standards of Thomson *et al.* (1968).

From the time they entered the study all of these pregnant women provided 24-hour samples of urine every two to three weeks, including the 24 hours before each infusion; the number of 24-hour samples ranged from 6 to 30 per patient. On the occasion of their non-pregnant (postnatal) infusion they again collected urine for the preceding 24 hours. Each 24-hour urine specimen was stored in a polythene bottle containing 5 ml. of 20 per cent aqueous chlorhexidine gluconate, which has been shown to prevent loss of glucose. The glucose content was determined by the hexokinase technique (as above) which is known to determine accurately urinary glucose as low as 5 mg. per 100 ml. (Lind *et al.*, 1971). In addition, every sample of urine passed during the week before the 24-hour urine collection was tested with "Clinistix" (Ames Company) in order to study the pattern of glycosuria. These reagent strips were of recent manufacture and kept in a tightly-closed screw-top bottle.

Measurements of glomerular filtration rate and tubular reabsorption of glucose were made at 15 to 18 weeks, 25 to 28 weeks and 35 to 38 weeks of pregnancy. Thirteen of the subjects were studied on three occasions, two were studied on two occasions and fourteen on one occasion during pregnancy. The measurements were repeated on all 29 subjects 8 to 12 weeks after delivery. Details are given in Table I.

A standard intravenous infusion technique was used (Davison and Cheyne, 1972) based on that described by Goldring and Chasis (1944). A priming infusion of 10 per cent dextrose containing 1 g. of inulin per 100 ml. was given by a constant infusion pump at a rate of 16 ml. per minute for 10 minutes. Thereafter a sustaining infusion of 10 per cent dextrose containing 0·5 g. of inulin was given for 20 minutes at 8 ml. per minute aimed to give plateau plasma levels of 150–250 mg. glucose per 100 ml. and 30 mg. inulin per 100 ml. The actual plateaux achieved are shown in Table I. When stable plasma levels had been achieved after 30 minutes, clearances were estimated for three successive 20 minute periods. A blood sample was taken at the midpoint of each such period. The women passed urine spontaneously.

| | | | | | Infant | | | Glucose excretion (mg./24h) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | During pregnancy | | | |
| Subject | Age | No. of previous pregnancies | Height (cm.) | Postnatal weight (kg.) | Birthweight (g.) | Gestation at delivery | Sex | No. days tested | Mean | Range | Po<br>na |
| *Non-glycosuric* | | | | | | | | | | | |
| JB | 30 | 1 | 164 | 51·3 | 3156 | 39 | F | 7 | 92 | 61–124 | |
| HC | 29 | 0 | 166 | 59·0 | 4330 | 41 | M | 12 | 149 | 96–197 | |
| ED | 34 | 4 | 168 | 62·7 | 3386 | 41 | F | 10 | 117 | 71–165 | |
| CC | 26 | 2 | 159 | 66·3 | 3480 | 37 | M | 9 | 98 | 67–168 | |
| KO | 29 | 3 | 164 | 55·0 | 3138 | 40 | M | 10 | 87 | 46–168 | |
| MS | 29 | 3 | 162 | 62·5 | 3628 | 39 | M | 8 | 146 | 92–366 | |
| YY | 32 | 1 | 160 | 53·7 | 3560 | 40 | M | 9 | 95 | 68–159 | |
| IB | 22 | 0 | 163 | 57·7 | 3572 | 39 | M | 6 | 116 | 98–142 | |
| KB | 29 | 3 | 171 | 64·1 | 3840 | 39 | M | 6 | 99 | 69–146 | |
| EY | 27 | 1 | 170 | 63·4 | 3954 | 40 | F | 8 | 134 | 96–174 | |
| DF | 29 | 4 | 159 | 61·5 | 2830 | 39 | M | 6 | 78 | 69–101 | |
| MH | 32 | 0 | 155 | 71·7 | 2880 | 39 | M | 8 | 118 | 94–158 | |
| IM | 27 | 1 | 156 | 51·5 | 3050 | 40 | M | 7 | 116 | 81–173 | |
| PC | 27 | 2 | 156 | 56·5 | 2900 | 39 | M | 30 | 82 | 54–130 | |
| *Moderate glycosuria* | | | | | | | | | | | |
| AB | 22 | 1 | 153 | 55·3 | 3636 | 40 | M | 10 | 165 | 75–336 | |
| SC | 29 | 2 | 156 | 61·0 | 3386 | 40 | F | 7 | 302 | 72–665 | |
| $JD_1$ | 26 | 0 | 158 | 52·9 | 3476 | 40 | M | 7 | 177 | 80–357 | |
| SE | 30 | 0 | 152 | 47·2 | 2460 | 38 | F | 12 | 436 | 63–959 | |
| JF | 28 | 1 | 155 | 48·9 | 3130 | 39 | F | 6 | 397 | 95–889 | |
| EK | 24 | 1 | 153 | 52·4 | 3500 | 39 | F | 6 | 328 | 182–596 | |
| EM | 28 | 2 | 164 | 52·0 | 3487 | 40 | M | 6 | 226 | 159–302 | |
| MR | 35 | 1 | 164 | 72·5 | 3704 | 40 | M | 6 | 299 | 108–492 | |
| CS | 26 | 0 | 174 | 65·4 | 3500 | 41 | F | 10 | 334 | 136–795 | |
| MA | 26 | 0 | 161 | 57·2 | 2480 | 40 | F | 6 | 522 | 302–992 | |
| $JD_2$ | 24 | 1 | 162 | 58·6 | 3450 | 40 | M | 7 | 225 | 61–390 | |
| IT | 31 | 0 | 173 | 66·8 | 3960 | 40 | F | 8 | 189 | 132–280 | |
| MG | 20 | 0 | 163 | 62·5 | 3500 | 39 | F | 6 | 255 | 130–411 | |
| *Heavy glycosuria* | | | | | | | | | | | |
| WH | 29 | 1 | 162 | 56·7 | 3660 | 39 | F | 8 | 10561 | 6600–19152 | |
| PW | 26 | 1 | 153 | 48·7 | 3100 | 38 | M | 8 | 9895 | 2424–23323 | |

The concentration of inulin in both urine and plasma was measured by a modification (Robertson *et al.*, 1970) of the technique of Heyrovsky (1956). Glucose in urine was determined by the hexokinase glucose-6-phosphate dehydrogenase method (Schmidt, 1963) using kits supplied by the Boehringer Corporation, and in plasma by a glucose oxidase/peroxidase method (Trinder. 1969).

Plasma insulin determination was by immunoassay, using a single antibody-charcoal separation system (Lind *et al.*, 1972).

## RESULTS

Detailed results are given in Table I.

### *Twenty-four hour urinary glucose excretion*

Every woman excreted more glucose in

dts

|  | Infusion studies |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  | GFR (ml./min.) |  |  | Plateau glucose (mg./100 ml.) |  |  |  | Mean insulin (μU./ml.) |  |  |  | Glucose reabsorption (per cent of filtered load) |  |  |  |  |
| 18 ks | 25–38 weeks | 35–38 weeks | Post-natal | 15–18 weeks | 25–28 weeks | 35–38 weeks | Post-natal | 15–18 weeks | 25–28 weeks | 35–38 weeks | Post-natal | 15–18 weeks | 25–28 weeks | 35–38 weeks | Post-natal |  |
| 0 | 162 | 149 | 86 | 128 | 143 | 136 | 165 | 124 | 123 | 192 | 76 | 80 | 73 | 77 | 80 |  |
| 4 | 166 | 167 | 103 | 132 | 148 | 222 | 196 | 107 | 132 | 278 | 79 | 93 | 90 | 84 | 98 |  |
| 3 | 153 | 142 | 96 | 139 | 179 | 217 | 162 | 95 | 73 | 84 | 33 | 84 | 82 | 83 | 97 |  |
| 8 | 198 | 197 | 147 | 261 | 247 | 258 | 234 | 100 | 106 | 212 | 52 | 60 | 66 | 78 | 81 |  |
| 3 | 214 | 186 | 124 | 254 | 199 | 196 | 292 | 28 | 37 | 27 | 27 | 68 | 79 | 71 | 80 |  |
| 5 | 151 | 146 | 84 | 116 | 162 | 183 | 200 | 70 | 73 | 191 | 42 | 85 | 76 | 82 | 98 |  |
| 3 | 201 | 201 | 135 | 220 | 229 | 253 | 208 | 80 | 71 | 80 | 74 | 65 | 78 | 68 | 98 |  |
|  | – | 203 | 105 | – | – | 222 | 190 | – | – | 328 | 33 | – | – | 84 | 97 |  |
| 1 | – | – | 137 | 132 | – | – | 180 | 70 | – | – | 38 | 89 | – | – | 91 |  |
| 9 | – | – | 119 | 117 | – | – | 160 | 80 | – | – | 35 | 91 | – | – | 97 |  |
|  | – | 171 | 101 | – | – | 155 | 198 | – | – | 53 | 73 | – | – | 97 | 95 |  |
|  | – | 201 | 120 | – | – | 158 | 156 | – | – | 111 | 36 | – | – | 90 | 99 |  |
|  | – | 203 | 95 | – | – | 243 | 178 | – | – | 353 | 68 | – | – | 78 | 89 |  |
|  | 150 | 137 | 99 | – | 176 | 205 | 175 | – | 113 | 308 | 43 | – | 87 | 78 | 99 |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 3 | 135 | 139 | 73 | 203 | 223 | 241 | 338 | 59 | 106 | 218 | 61 | 65 | 64 | 64 | 64 |  |
| 3 | 175 | 179 | 77 | 136 | 134 | 162 | 162 | 131 | 128 | 161 | 52 | 80 | 76 | 81 | 95 |  |
|  | 187 | 169 | 100 | 267 | 203 | 207 | 320 | 52 | 114 | 89 | 62 | 64 | 75 | 75 | 75 |  |
| 5 | 158 | 157 | 108 | 262 | 293 | 267 | 360 | 63 | 51 | 121 | 51 | 58 | 45 | 47 | 65 |  |
|  | 174 | 166 | 114 | 262 | 154 | 281 | 261 | 103 | 145 | 166 | 48 | 55 | 49 | 50 | 66 |  |
|  | – | 156 | 94 | – | – | 203 | 214 | – | – | 134 | 46 | – | – | 58 | 69 |  |
|  | – | 162 | 93 | – | – | 188 | 260 | – | – | 257 | 64 | – | – | 55 | 66 |  |
|  | – | 183 | 122 | – | – | 195 | 164 | – | – | 168 | 59 | – | – | 51 | 81 |  |
|  | – | – | 126 | 153 | – | – | 233 | 105 | – | – | 60 | 76 | – | – | 79 |  |
|  | – | 173 | 111 | – | – | 156 | 174 | – | – | 204 | 59 | – | – | 80 | 85 |  |
|  | – | 151 | 95 | – | – | 213 | 205 | – | – | 114 | 83 | – | – | 81 | 81 |  |
|  | – | 164 | 145 | – | – | 197 | 234 | – | – | 249 | 95 | – | – | 72 | 76 |  |
|  | – | 134 | 92 | – | – | 223 | 165 | – | – | 430 | 75 | – | – | 61 | 77 |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 195 | 192 | 90 | – | 196 | 214 | 210 | – | 106 | 137 | 68 | – | 42 | 44 | 61 |  |
|  | 135 | 118 | 80 | 245 | 262 | 228 | 335 | 101 | 86 | 157 | 30 | 34 | 34 | 45 | 53 |  |

pregnancy than at 8 to 12 weeks after delivery. The losses varied widely between women, and from time to time in a given woman during pregnancy but in every instance the excretion of glucose in the postpartum 24-hour samples were all within normal limits.

For the purpose of analyzing the infusion data we have divided the women into groups according to their 24-hour urinary glucose excretion during pregnancy. They were designated "non-glycosuric" if they never excreted more than 150 mg. glucose in 24 hours and "glycosuric" if they regularly excreted more than 150 mg. in the 24-hour periods. The "glycosuric" group was further subdivided into those with "moderate glycosuria" if up to 600 mg. per 24 hours was exceeded on not more than one occasion, and those with "heavy glycosuria" if it exceeded

600 mg. on several occasions. The divisions are arbitrary and we recognize that the highly irregular nature of glucose excretion could readily lead to mis-classification of any given women.

"Clinistix" testing revealed that the women with both "moderate" and "heavy" glycosuria had intermittent "positives" throughout pregnancy, in an irregular pattern similar to that described by Lind and Hytten (1972).

### *Reabsorption of glucose during infusion*

The mean GFR, the mean plateau of glucose concentration, the mean insulin level and the mean percentage of the filtered load which was reabsorbed are given in Table I. The three clearance periods which contributed to the mean value for each infusion showed no trend except that at plateau levels of glucose above 200 mg. per 100 ml. there was a tendency for the percentage reabsorption to decline progressively.

There was no apparent change during the course of pregnancy in the major variables measured. That is to say, the GFR, the plateau level of plasma glucose and the percentage reabsorption tended to remain at levels characteristic of the individual pregnancy throughout

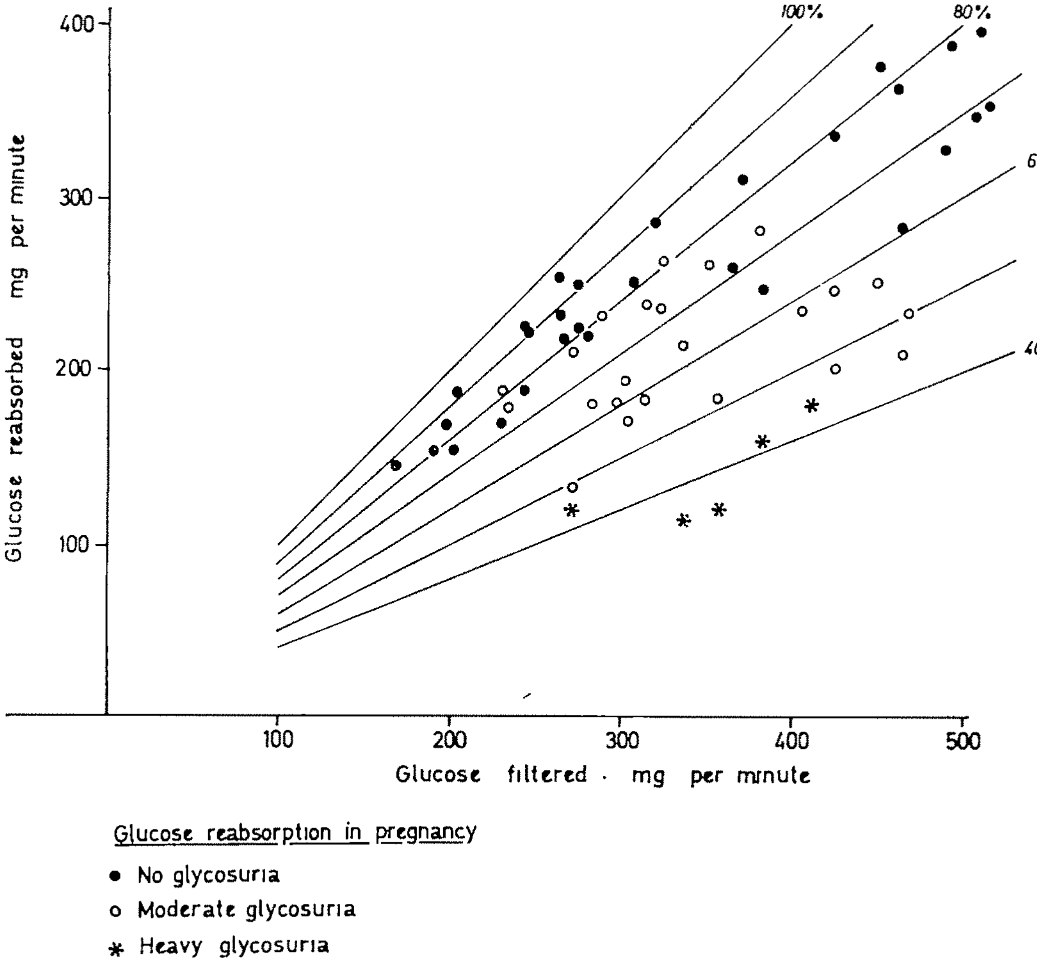

FIG. 1

The relation of the amount of glucose filtered (mg. per minute) to the amount reabsorbed (mg. per minute) in 57 infusion tests in 29 pregnant women: those without glycosuria, those with "moderate" glycosuria and those with "heavy" glycosuria. For definitions see text. The radiating lines show levels of percentage reabsorption.

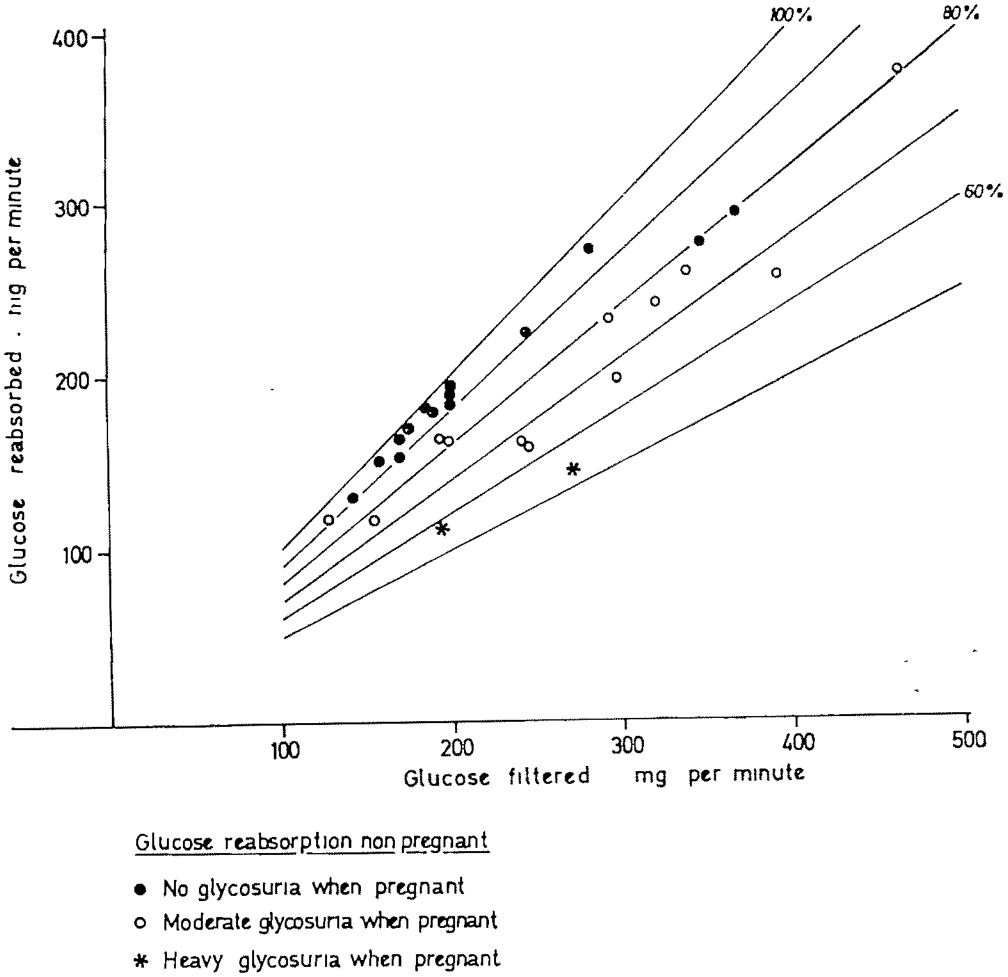

FIG. 2

The relation of the amount of glucose filtered (mg. per minute) to the amount reabsorbed (mg. per minute) in the three groups of women 8 to 10 weeks after delivery, classified in terms of whether or not they had glycosuria during the preceding pregnancy. For definition see text. The radiating lines show levels of percentage reabsorption.

the period studied. There was a tendency, however, significant at the 5 per cent level, for the plateau plasma glucose level to be higher in the glycosuric group during the postnatal infusion; four of the fifteen glycosuric women had plateau glucose concentrations in excess of 300 mg. per 100 ml., a level which was not seen in the non-glycosuric group, nor in any subject during pregnancy.

The only conspicuous change during the course of pregnancy was the dramatic rise demonstrated by some but not all subjects in mean insulin level during the glucose infusion. This was not related to the phenomenon of glycosuria.

In Figures 1 and 2 the amount of glucose reabsorbed per minute is plotted against the amount filtered per minute during the infusion. The filtered load is calculated as the glomerular filtration rate multiplied by the plateau concentration of glucose; the amount reabsorbed is the filtered load less the quantity excreted in the urine per minute.

Comparison of the two diagrams reveals that the capacity of the kidneys to reabsorb glucose is usually reduced in pregnancy, whether or not the woman exhibits glycosuria. But the capacity of the individuals to reabsorb glucose *in the non-pregnant state* is related to the degree of glycosuria they exhibited in pregnancy, even though all subjects were, at that stage, no longer excreting amounts in excess of 100 mg. per 24 hours (Fig. 2).

Thus, the average woman who has no significant glycosuria in pregnancy will *during pregnancy* reabsorb on average between about 70 and 90 per cent of a filtered load of between 200 and 350 mg. per minute, whereas *after pregnancy* she will generally reabsorb in excess of 90 per cent of a similar filtered load.

A woman with moderate glycosuria in pregnancy will *during pregnancy* absorb only between 50 and 80 per cent of the filtered load and even *after pregnancy* she will still reabsorb only about 65 to 85 per cent. The two women with gross glycosuria reabsorbed around 40 per cent of the filtered load in pregnancy but were still unable to reabsorb more than about 60 per cent when not pregnant.

## DISCUSSION

In normal non-pregnant circumstances the tubular reabsorption of glucose is highly efficient; even women who have gross glycosuria during pregnancy can, when they are not pregnant, excrete less than 150 mg. in 24 hours and since they will have filtered some 150 g. in that time the efficiency of reabsorption is better than 99·9 per cent.

Pregnancy therefore appears to be an exceptional circumstance which uncovers, in a large number of women, an inability to reabsorb glucose which becomes strikingly apparent under the conditions of glucose infusion.

To a minor extent variables associated with the infusion itself may have blurred the results. Both the volume of the infusion and the plasma glucose level attained exercise a small influence on GFR and glucose reabsorption, and we are currently developing techniques whereby plasma glucose levels can be kept constant and below 200 mg. per 100 ml. with a constant intravenous fluid load. But the gross differences in glucose reabsorption between pregnant and non-pregnant subjects cannot be attributed to minor inconsistencies of infusion technique. Pregnancy itself must therefore impose some specific change in the reabsorptive capability of the proximal tubule.

What remains to be explained is the characteristic intermittency of clinical glycosuria in pregnancy which bears little relation to meals and hence to plasma glucose concentrations (Lind and Hytten, 1972). The basic effect of pregnancy on the proximal tubules, whether hormonal or the results of some other change in *milieu interieur*, is unlikely to fluctuate with the intermittency described for glycosuria. Is it likely, then, that a variable filtered load and the consequent variable proximal tubular exposure could cause episodic glucose excretion?

During a normal day overall renal plasma flow and GFR fluctuate more than physiological measurements under standard conditions would suggest (Wesson, 1964), but even with overall stability, the distribution of blood flow within the kidney is varying from minute to minute so that any group of nephrons will be subject to a continually varying plasma flow and filtration rate. Moreover, this shifting distribution of blood flow within the kidney is to nephron groups which are themselves anatomically heterogenous and seem likely to vary in performance (Bradley and Wheeler, 1958; Oliver and MacDowell, 1961; Gottschalk, 1969).

In the normal non-pregnant woman plasma flow to each glomerulus and the proportion filtered is much less than the maximum possible and in general all nephrons are "ticking over" at something well below the capacity of the best and within the capability even of the least efficient.

The effect of pregnancy in raising the renal plasma flow and GFR by from 50 to 100 per cent will completely alter the picture; while nephrons with a large reabsorptive capacity will still be capable of dealing with the increased filtered load, the nephrons with lesser capacity will fail to cope in varying degree, and their contribution to total renal function at any given time will depend on the distribution of the blood flow within the kidney at that time. The greater the number of less efficient proximal tubules the greater the degree and persistence of glycosuria, and their contribution to total renal function at any given time will result in intermittency of glycosuria. Glucose infusion may result in all nephrons being maximally perfused, and the inadequacies of the less efficient tubules are fully exposed.

Such a postulate supposes some individuals have a significant proportion of inadequate tubules. It is known that there is diminished renal tubular function in infants following vascular damage (Stark and Geiger, 1972) and Monasterio *et al.* (1964) have demonstrated "congenital" anatomical defects in the tubules of persons with "renal glycosuria". In our experience the phenomenon of reduced glucose reabsorption is not apparent in healthy young men, so that the explanation may lie in a condition restricted to a proportion of apparently healthy young women; the most obvious possibility is damage from renal infection in childhood or adolescence. Minor degrees of damage even from asymptomatic renal infection may be common in young girls and a group of carefully documented cases is currently under study. And since it is unlikely that only glucose reabsorption is affected, the reabsorption of other nutrients will be examined.

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# FETAL DEATH IN ECLAMPSIA: I

## ITS RELATION TO LOW GESTATIONAL AGE, RETARDED FETAL GROWTH AND LOW BIRTHWEIGHT

BY

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### Summary

Of 173 eclamptics admitted to the only public maternity hospital in Cali, Colombia 44 had stillbirths making a rate of 25·4 per cent. Fetuses delivered before 32 weeks had nearly four times the stillbirth rate of those delivered at term. Those weighing less than 1600 g. had stillbirth rates about six times higher than those weighing above 2500 g. An attempt was made to divide the risk conveyed by low birthweight into two components; that which was due to low gestational age, and that which was due to an abnormally low rate of growth during the gestational time available. Deviation of birthweight below that expected for age (retarded fetal growth, RFG) was used as a measure of the latter component. RFG became more common as gestation progressed and was associated with fetal death only in the latter part of gestation and with marginal statistical significance. After 35 weeks, fetuses at or below two standard deviations from Gruenwald's mean weight for gestational age (Gruenwald, 1966) had a fetal death rate five times higher than those at the mean. The risk of fetal (intrauterine) death conveyed by low gestational age has not been definitively explained. Alternative pathophysiological mechanisms are discussed.

A NUMBER of authors have noticed that low gestational age or low birthweight carries a high risk of fetal death in pre-eclampsia (Drillien, 1947; Brash, 1949; Hendricks *et al.*, 1971; Taylor *et al.*, 1954; Gruenwald, 1966) and eclampsia (Tunis, 1928; Peckham, 1933; Dieckmann, 1952; Tyson and Bonman, 1931; Zuspan, 1966; Corckhill, 1961; Lopez-Llera and Hernandez, 1972). These articles, however, were mostly written before it was recognized that low birthweight can occur in two ways: one, by interruption of the fetus' growing time, (low gestational age), and the other, by an abnormally low rate of growth during the gestational time available (retarded fetal growth, RFG) (Lubchenco *et al.*, 1963; Gruenwald, 1969). It has been observed that RFG babies at term suffer different kinds of neonatal morbidity than do low gestational age babies of the same weight (Gruenwald, 1969). Gruenwald (1969) has shown that the *in utero* mortality for English fetuses, even at term, increased as their weight fell below the expected value for that gestational age.

The presence of RFG has been studied in pre-eclampsia (Hendricks, 1971; Baird *et al.*, 1957) and eclampsia (Lopez-Llera *et al.*, 1972). The risk of fetal death which RFG carries has been documented for pre-eclamptic (Hendricks, 1971) and hypertensive (Gruenwald, 1966) pregnancies but to date it has not been documented in eclampsia. Because of this, and because eclampsia is still an important problem in Cali, Colombia, where this study was done, it seemed useful to examine separately the impact of gestational age, retarded fetal growth and the resultant birthweight on the fetal death rate in eclampsia. A companion article discusses the impact on the fetal death rate of other factors which have been known to predict eclampsia.

## METHODS

Cali, Colombia is in many ways typical of cities in developing countries. Its population has grown from 88 000 in 1938 to 1 million in 1972. Twenty per cent of its citizens live without benefit of running water, sewerage or paved streets, and malnutrition is common. Of the 30 000 births which occurred in 1969, 50 per cent were at home. Nonetheless, as has been explained in a previous publication (Neutra, 1973) there is good reason to believe that nearly all patients with eclampsia receive hospital care. A total of 220 eclamptics with single births were admitted to the only public maternity hospital in Cali between 1st January 1964 and 31st December 1970.

In this group of 220, there were 17 maternal deaths (7·7 per cent) and 54 fetal deaths (24·5 per cent). Only the eclamptic women with full information on variables shown to predict eclampsia itself (age, parity, marriage status, abortion history) were selected for analysis. This eliminated 10 patients with stillbirths, 24 patients with liveborn infants and 13 patients for whom no information, not even about survival of the infant, was recorded.

The analysis therefore consisted of comparing the medical records of 44 patients with stillbirths with those of 129 patients whose babies were born alive. This part of the analysis examines the importance of gestational age, retarded fetal growth and birthweight.

### *Definition of variables studied*

Gestational age and birthweight were taken directly from the patient's medical record. Retarded fetal growth (RFG) was measured using a weight deviation score developed by Gruenwald (1969). The mean birthweight for fetuses of the same age and sex as the subject ($\overline{\mathrm{BWT}}$) was subtracted from the observed birthweight (BWT). This difference was divided by the standard deviation (SD) for that age-sex group:

$$\text{“Gruenwald's score”} = \frac{\mathrm{BWT}-\overline{\mathrm{BWT}}}{\mathrm{SD}}$$

For example, a fetus 2 standard deviations below the expected mean has a score of $-2$, while a fetus 2 standard deviations above the mean has a score of $+2$. It should be noticed that the score becomes more negative as RFG increases. This study uses the means and standard deviations based on the British data obtained by Gruenwald (1969). There are no equivalent Colombian data available.

Technically, fetal death in this study should mean that there were no signs of life in the fetus at delivery. In fact this judgment was made by a number of different interns and residents, and it is likely that some of these fetuses really died within a few minutes after birth. This problem would be more marked for the very small fetuses, who would be assumed nonviable. It is not likely that the entire association between birthweight and fetal death could be explained on that basis. The record system in the hospital was such that a baby who was sent to the intensive care nursery and subsequently died could not be traced. The liveborn infants in this study thus include an unknown number of early neonatal deaths.

### *Analysis*

Trends in the death rates by gestational age, birthweight and Gruenwald Score were tested by Chi square for linear trend (Snedecor and Cochran, 1967). When the P value for this Chi square was below 0·05, we concluded that the slope of the death rates was significantly different from zero. The Mantel Normal Deviate was used to test the trend of death rate with the Gruenwald Score averaged over gestational age (Mantel and Haenszel, 1959). In these latter calculations only the survivors and stillbirths with complete information on the relevant variables were used. A linear discriminant function was used to observe the independent contributions of these three variables to fetal death. The Mahalanobis $D^2$ statistic was used to compare the discriminating power of the several possible discriminant functions (Rau, 1952). In this analysis all 129 survivors and 44 stillbirths were used. In 9 per cent of the stillbirths (4 patients) and 12 per cent of the survivors (15 patients) there were no data on gestational age or birthweight. If either variable was unknown, the mean gestational age of the entire series, 36 weeks, was substituted as well as the expected birthweight for that age; this gave a Gruenwald score of zero. Since the missing values occurred with about equal frequency in stillbirths and survivors, it is not thought that these substitutions introduced any important artefacts to the analysis.

In the following pages the term "prediction" is occasionally used to describe the relationship between Gruenwald score and birthweight on the one hand and fetal death on the other. Of course birthweight and Gruenwald score can only be known after delivery and thus cannot "predict" fetal death before the fact. But, *after the fact*, knowledge of the birthweight and Gruenwald scores from two differently treated groups of eclamptic women would allow one to "predict" their expected fetal death rate. This allows one to separate any effect of treatment from the effect produced by differences in the two groups' birthweight or Gruenwald score. It is in *this* sense that the word "predictor" is used here.

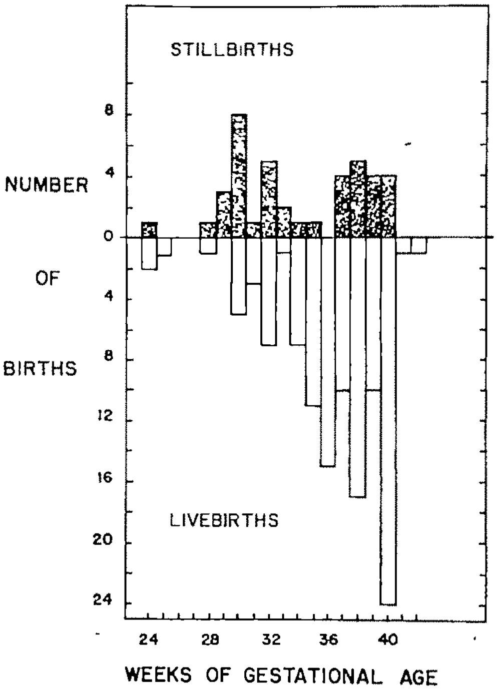

FIG. 1

A greater proportion of the eclamptic babies who were delivered before 35 weeks were stillborn.

RESULTS

*Gestational age*

Figure 1 shows the distribution of stillbirths and livebirths according to gestational age at birth. Stillbirth rates were higher when eclampsia (and delivery) occured earlier in pregnancy. Table I shows that the association of fetal death rate with gestational age is statistically significant. Before 32 weeks rates were about four times higher than at 40 weeks or after. It is interesting to note that by 36 weeks, when many antenatal patients begin weekly visits, 57 per cent of the fetal deaths have already occurred.

TABLE I

*Fetal death rates by gestational age at birth*

| Gestational age (weeks) | No. of stillborn infants in a category | No. of liveborn infants in a category | Stillbirth rate (per cent) |
|---|---|---|---|
| 24–31 | 15 | 11 | 57·7 |
| 32–35 | 9 | 26 | 25·7 |
| 36–39 | 14 | 52 | 21·2 |
| 40+ | 4 | 26 | 15·4 |
| Total* | 42 | 115 | 26·8 |

$X^2$ for Linear Trend = 13·34 1 d.f. P<0·001.

* Gestational age not known for 2 stillbirths and 14 livebirths.

*Birthweight*

Table II demonstrates a decreasing risk of fetal death as birthweight increased. Fetuses of less than 1600 g. had about six times the stillbirth rate of fetuses above 2500 g. This relationship, too, is statistically significant.

TABLE II

*Fetal death rate by birthweight*

| Birthweight (g.) | No. of stillborn infants in a category | No. of liveborn infants in a category | Stillbirth rate (per cent) |
|---|---|---|---|
| <1000 | 8 | 1 | 88·9 |
| 1001–1599 | 9 | 8 | 52·9 |
| 1600–2199 | 8 | 32 | 20·0 |
| 2200–2499 | 8 | 14 | 36·4 |
| 2500+ | 7 | 59 | 10·6 |
| Total* | 40 | 114 | 26·0 |

$X^2$ for Linear Trend 24·1 1 d.f. P<0·001.

* Birthweight not known for 4 stillbirths and 15 livebirths.

TABLE III
*Fetal death rate by Gruenwald score controlling for gestational age*

| Gruenwald score | Gestational age | | | | | |
|---|---|---|---|---|---|---|
| | Less than 35 weeks | | | 35 weeks and over | | |
| | No. of survivors in a category | No. of stillborn infants in a category | Stillbirth rate (per cent) | No. survivors in a category | No. of stillborn infants in a category | Stillbirth rate (per cent) |
| $>0$ | 6 | 8 | 57 | 23 | 2 | 8 |
| $-0{\cdot}99$ to 0 | 15 | 5 | 25 | 22 | 6 | 20 |
| $-1{\cdot}99$ to $-1{\cdot}00$ | 3 | 7 | 70 | 35 | 5 | 13 |
| $\leqslant -2{\cdot}00$ | 1 | 1 | 50 | 9 | 6 | 40 |
| Total* | 25 | 21 | 46 | 89 | 19 | 18 |

| Components of $X^2$ | d. f. | Value | P | Value | P |
|---|---|---|---|---|---|
| Linear | 1 | ·123 | n.s. | 3·23 | ~·06 |
| Dev. | 2 | 6·46 | <·05 | 4·55 | ~·10 |
| Total | 3 | 6·58 | n.s. | 7·78 | ~·06 |

Mantel normal deviate = 1·66, P = 0·048 (one tail)

* 4 Stillbirths, 15 survivors with missing data.

*Retarded fetal growth*

The Gruenwald score, when taken by itself, did not predict fetal death. Gruenwald (1969) has pointed out that the impact of RFG (retarded fetal growth) on fetal death is usually noticed at or after the 35th week of gestation. In Table III, therefore, we have controlled for gestational age and distributed the fetuses according to their Gruenwald score—the number of standard deviations by which they differed from their expected weight. Before 35 weeks of gestation, the fetal death rate was high (46 per cent), but did not seem to vary with the Gruenwald score. After 35 weeks the overall rate was lower (18 per cent), yet a negative score now conveyed excess risk. Fetuses at or below —2 standard deviations had five times the death rate of fetuses at or above the mean. The Chi square for linear trend does not quite reach the 0·05 level of significance for this part of Table III, but the p value for the Mantel Normal Deviate, which is testing the trend for the entire table regardless of gestational age, is significant in a one tail test.

Figure 2 relates the mean weight at each gestational age of the eclamptic survivors (white circles) and of the stillbirths (black circles) to the curve of mean weights for age for Gruenwald's British infants. Circles for survivors and stillbirths in the same age group are connected by a line. This provides a more detailed picture of the data which produced Table III. Again, it is clear that after 35 weeks there was a tendency

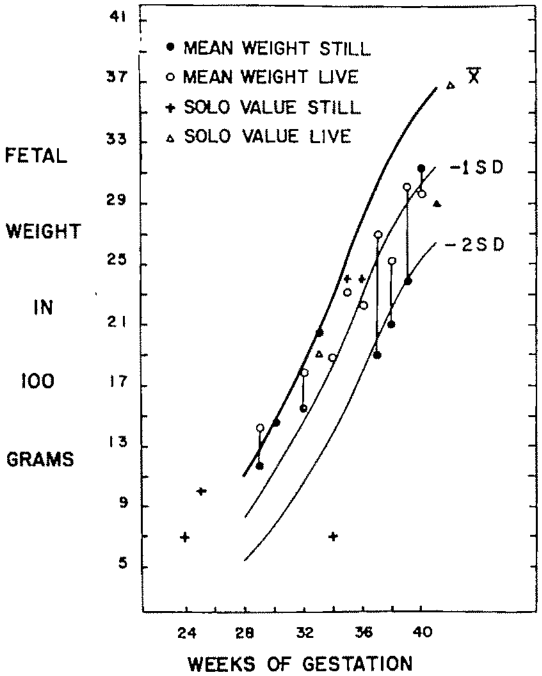

FIG. 2

Mean weight, at each gestational age, of survivors (white circles) and stillbirths (black circles) are related to the curve of expected mean weights for age. Circles for survivors and stillbirths in the same age group are connected by a line. When only one measurement was available in a category ("solo value") a cross or a triangle is used for stillbirth or livebirth, respectively. After 35 weeks there was a tendency for stillbirths to have birthweights below those of livebirths at the same gestational age. About 75 per cent of fetuses whose mothers had eclampsia had weights at or below that expected for their gestational age (see also Table III).

for stillbirths (black circles) at a given age to have birthweights below those of the live births (white circles). Figure 2 and Table III also demonstrate that eclamptic fetuses, stillborn or alive, were smaller than the average for British infants. This retardation became more marked as gestation progressed. Calculation from the numbers in Table III shows that the 69·5 per cent of eclamptic fetuses born before 35 weeks and 76·9 per cent of eclamptic fetuses delivered thereafter had a birthweight below the British mean. Similarly, 4·3 per cent and 13·9 per cent respectively were two or more standard deviations below the mean. In Gruenwald's standard series only about 3 per cent of the total population fell at or below this level (1969).

### *A multivariate analysis of the independent contributions of gestational age, birthweight and "Gruenwald score"*

Table IV shows the Mahalanobis $D^2$ for each of the above variables, both separately and in combination, as predictors of fetal death. The bigger the value of $D^2$ the better the prediction. It can be seen that birthweight alone is a better predictor than gestational age or Gruenwald score alone. The combination of gestational age and birthweight, or gestational age and Gruenwald score is somewhat better than birthweight alone, although this difference is not statistically significant. Table V shows the coefficients and "t" tests for the latter and former formulae. It can be seen that the coefficient for gestational age is not significant in a formula which simultaneously includes birthweight but that both gestational age and Gruenwald score have significant coefficients when included in the same formula. Gestational age is too strongly correlated with birthweight for the discriminant function to demonstrate any independent contribution, but the gestational age is less correlated with deviations from expected weight and thus both terms' contributions can be estimated. The latter formula gives a clear cut corroboration of the findings in Table III: gestational age and deviations from expected weight are independently associated with the fetal death rate. The information obtained from these two variables, as judged from the Mahalanobis $D^2$, however, is only slightly greater than that gained by knowing birthweight alone.

TABLE IV

*Mahalanobis $D^2$ values for assessing the isolated and combined power of gestational age, birthweight and Gruenwald score as "predictors" of fetal death*

| Variables | Mahalanobis $D^2$ |
|---|---|
| Gruenwald Score alone | 0·041 |
| Gestational age alone | 0·497 |
| Birthweight alone | 0·729 |
| Gestational age + Gruenwald Score | 0·732 |
| Birthweight + gestational age | 0·741 |
| Birthweight + Gruenwald Score | 0·741 |
| All three variables | 0·741 |

TABLE V

*Two discriminant functions for "predicting" fetal death*

| Variable | Coefficient | t (169 d.f.) |
|---|---|---|
| (1) | | |
| Gestational age | $-2\cdot8\times10^{-4}$ | $-0\cdot595$ |
| Birthweight | $-6\cdot2\times10^{-6}$ | $-2\cdot623$ |
| (Mahalanobis $D^2=0\cdot741$) | | |
| (2) | | |
| Gestational age | $-1\cdot5\times10^{-3}$ | $-4\cdot416$ |
| "Gruenwald score" | $-2\cdot8\times10^{-3}$ | $-2\cdot573$ |
| (Mahalanobis $D^2=0\cdot732$) | | |

## DISCUSSION

In this group of eclamptic patients, low gestational age, retarded fetal growth (RFG) and low birthweight were common. Birthweight was the strongest single predictor of fetal death. Alone it was almost as good a predictor of fetal death as the combination of birthweight and gestational age or the combination of Gruenwald score and gestational age. In fact, the added predictive power afforded by these two combinations was not statistically significant in this small series. A finding similar to this has been emphasized by Susser *et al.* (1972) in his study of perinatal death in the general population. He showed that birthweight alone was almost as good a predictor of the total perinatal death rate as both birthweight and gestational age together. In his large series, however, the information added by gestational age was statistically significant. This was particularly marked towards the end of gestation.

Despite these findings it seems useful to continue making the distinction between babies who are small due to low gestational age and babies who are small due to retarded fetal growth. The distinction certainly has been useful in the early neonatal period when low gestational age babies have a high mortality from hyaline membrane disease while RFG babies have other problems and a better overall prognosis (Gruenwald, 1969; Van den Berg and Yerushalmy, 1966). It is not unreasonable to expect that *in utero* as well as neonatally, the two kinds of fetuses die for different reasons and that prevention and treatment may be different for the two. Therefore, in this article and in its companion the author has elected to emphasize separately both gestational age and RFG even though from a statistical point of view the resultant birthweight would predict just as well.

Ludlow (1933), Drillien (1947) and Brash (1949) pointed out that toxaemic babies had a better neonatal prognosis than non-toxaemic babies of the same weight. The findings in this study suggest that many of their babies may have been small due to RFG and not due to low gestational age. The high proportion of these RFG babies, with their better neonatal prognosis (Van den Berg and Yerushalmy, 1966) would have given toxaemic babies an apparent advantage. If the authors had controlled for gestational age, this advantage might have disappeared.

It seems unlikely that the association between fetal death and low birthweight or RFG could be due to the presence of macerated fetuses who had lost weight *in utero* after their death. Of the fetuses who were alive on admission, but died subsequently, most were delivered within 24 hours and all were delivered within 72 hours of their death. Seven fetuses were dead on arrival and the time of their death was not known. If one assumes that all were macerated and removes them from the analysis, the direction of the risk conveyed by low Gruenwald score or low birthweight still persists. As a matter of fact only one of the seven had a Gruenwald score below −2 SD so that most, if not all of them, were probably expelled rather soon after death.

There may be several explanations as to why the stillbirth rate should be higher if eclampsia strikes early in gestation: (1) the gestationally young fetus is somehow less able to withstand the toxaemic process or the toxaemic therapy, (2) the syndrome of eclampsia superimposed on hypertension, which is known to have high fetal mortality rates, tends to occur earlier in pregnancy, (3) the prognosis of any fetus whose mother goes into premature labour is poor and eclamptic fetuses are no different. Knowing the true explanation might influence the choice of medication in early onset eclampsia. the differential treatment of eclampsia superimposed on hypertension, or the choice of caesarean section versus vaginal delivery for early onset eclampsia. It seems to the author that not enough attention has been devoted to this question and, although this study cannot give a definitive answer, some speculation is justified since the data suggest that no single one of the above explanations is sufficient to account for the entire phenomenon.

If the risk conveyed by low gestational age was due to a particular vulnerability of the premature fetus to the toxaemic process or toxaemic medications, the association would be most marked for antepartum deaths. In this series, because of the uncertainty surrounding the temporal relation of death to the onset of labour, the proportion of antepartum deaths may vary from 30 to 50 per cent. We therefore cannot examine the relationship between gestational age and antepartum death rates. Unfortunately, Hendricks (1971) and Lopez-Llera and Hernandez (1972) did not address this issue either, so that it is a question which remains to be answered.

If the entire gestational age effect was due to the early occurrence of "eclampsia superimposed on hypertension" we would expect RFG (common in hypertension) to be most marked early in the third trimester and to see little gestational age effect in primiparae (who rarely have underlying hypertension). In fact, RFG became more common later in gestation, and primiparae displayed a strong gestational age effect, too. We will return to this point in the companion article (Neutra and Neff, 1975).

The third explanation proposes that the fate of *any* fetus whose mother goes into premature labour would be poor and that premature fetuses of eclamptics are no different. Some authors come to this view by physiological reasoning, and others (Drillien, 1947; Tunis, 1928) through statistical analysis. A physiological explanation,

TABLE VI

*Percentage distribution of stillbirths and survivors as to gestational age and labour length*

| Length of labour | Gestational age | Survivors in a category (per cent) | Stillborn infants in a category (per cent) | Stillbirth rate* (per cent) |
|---|---|---|---|---|
| 0–9 hours | 24–30 weeks | 3·0 | 14·7 | 62·2 |
| | 31–35 weeks | 25·8 | 2·9 | 3·7 |
| | 36–39 weeks | 35·5 | 3·2 | 23·8 |
| | 40+ weeks | 16·1 | 0 | 0 |
| 10 hours or more | 24–30 weeks | 3·0 | 3·8 | 50·0 |
| | 31–35 weeks | 0 | 20·5 | 100·0 |
| | 36–39 weeks | 9·7 | 11·8 | 29·3 |
| | 40+ weeks | 6·5 | 8·8 | 31·6 |
| Total† | | 100·0<br>n = 31 | 100·0<br>n = 34 | Overall rate 25·4 |

$X^2$ (0–9 hours) = 12·02; $p<0·01$; 3 d.f.
$X^2$ (10 hours or more) = 3·99; p n.s.; 3 d.f.
Mantel normal deviate = 2·00 p (Two tailed)$\sim$0·05 1 d.f.

* Rates calculated by extrapolating percentages to fill number of survivors.

† Missing information on labour length and gestational age.

once current but criticized by Zuspan (1966), was that the cervix in premature labours is unripe and the labour prolonged and traumatic. Table VI compares the stillbirths to a random subsample of the survivors, as to prematurity, controlling for length of labour. The numbers are small, but the figures suggest that low gestational age continues to exert its influence regardless of labour length. Thus, the cervix in premature labour cannot be the entire explanation.

The authors who used statistics to justify the third explanation (Drillien, 1947; Tunis, 1928) compared the eclamptic fetal death rates to the rates in non-toxaemic women and found that early in pregnancy, the rates were equally high in both groups. Indeed, before 35 weeks of gestation, the eclamptic women's rates in *this* study were about the same as the rates reported by Gruenwald (1969) for a random sample of all births in England But women who went spontaneously into premature labour probably did not constitute a valid comparison group. They went into premature labour because of a variety of underlying pathological conditions, such as malformation, which caused both prematurity and fetal death. They themselves were abnormal and their fetal death rate was therefore higher than the rate that would be expected for the women in this study if their eclampsia had been prevented but premature labour induced. Comparing toxaemic and non-toxaemic fetal death rates early in pregnancy can thus be misleading. In fact, authors who have made such comparisons (Drillien, 1947; Brash, 1949; Hendricks, 1931; Tunis, 1928) have come up with conflicting conclusions, probably because of the differing degree of abnormality in the comparison groups used. This is not to deny, however, that the intrapartum "fragility" of premature fetuses may account for some of the gestational age effect in eclamptic fetal death. Lopez-Llera and Hernandez (1972) have even presented data which suggest that Caesarean section can avoid traumatic labour and save fetal lives.

Taylor *et al.* (1954) have studied the relative fetal risk of inducing labour or prolonging gestation in pre-eclampsia. In eclampsia most women go into prompt spontaneous labour anyway (70 per cent in this series). In those who do not, the threat of maternal mortality usually impels induction and/or Caesarean section. The data in this study remind us that the fetus whose mother develops eclampsia early in the third trimester has a higher risk *in utero* than he would if his mother developed eclampsia at term. Hopefully, the obstetrician can improve his situation by making his antepartum or intrapartum environment safer without imperilling his mother. The fact that 75 per cent of the dead fetuses in this study had not yet died one hour after their mothers' admission suggests that there is still time for obstetrical intervention on their behalf.

## ACKNOWLEDGEMENTS

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# FETAL DEATH IN ECLAMPSIA: II

# THE EFFECT OF NON-THERAPEUTIC FACTORS

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## Summary

The ability of 15 variables to predict fetal death is examined among 173 eclamptic women admitted to the only public maternity hospital in Cali, Colombia, between 1st January 1964 and 31st December 1970. In addition to low gestational age and retarded fetal growth, high systolic pressure and the unmarried status carried excess risk. Primiparae appeared to be of lower risk because their eclampsia tended to occur late in gestation and was characterized by less retarded fetal growth. Older women and women with a history of abortion appeared to be of higher risk because they tended to have higher systolic pressures. A discriminant function risk formula is presented which generated groups with a nine-fold difference in fetal death rates. This formula could be used to standardize for relevant non-therapeutic factors which might vary between patient groups who had received different therapeutic regimens.

VARYING rates of fetal survival have been reported for eclampsia in recent years (Zuspan and Ward, 1965; Pritchard and Stone, 1967; Lopez-Llera and Hernandez, 1972), indicating on the one hand the promise of new eclamptic regimens and on the other the presence of non-therapeutic factors, such as the type of eclampsia and the age of the patients, which influence the prognosis of the fetus. It would be helpful when comparing the results from one institution to the next or in comparing results in one institution over time, to have a way to standardize for relevant non-therapeutic factors which may vary from one patient series to the next. With this aim in mind and also with the goal of clarifying the epidemiology of eclamptic fetal deaths it was thought useful to examine the interrelationships and independent contributions of 15 non-therapeutic factors suspected of predicting or correlating with fetal death.

## METHODS

The 173 eclamptics studied in this article are the same women studied by Neutra (1975); details of the environment, selection and composition of the patients are given in that paper.

### *Variables studied*

The information on all variables was obtained retrospectively from routine medical records at the University Hospital in Cali, Colombia.

1. Primiparity: Since only primiparae were at excess risk of eclampsia, the variable was dichotomized. Women having their first baby were coded as "1", multiparae were coded as "0".
2.–4. Age, history of previous abortions, history of previous stillbirths.
5. Marital status: Hospital records in Cali distinguish between two kinds of unmarried women: spinsters and women in free union. In practice, however, this distinction is often unreliable. Thus, for this study we have simply designated the women as married or unmarried. The latter group includes a few widows and separated women. It must be stressed that we are measuring the stated marriage status which may or may not conform to the legal marriage status.
6. Social status: This has been assessed on the basis of the neighbourhood ("barrio") where the patient or control resided. The city planning department has ranked barrios on a scale from low "1" to a high "9" based on property values, extent of street paving and sewage lines and amount of water and electricity consumed. The address on the hospital chart was used to assign a rough socioeconomic rank to each woman in the study.
7. Birth place: This was used as a crude indicator of migration to Cali and was coded: born in Cali, born in the State of Valle, born elsewhere in Colombia.
8.–9. Blood pressure: The highest antepartum diastolic pressure during the hospitalization was used, as well as the systolic pressure which accompanied it.
10. Proteinuria: The highest "albustix" measure of proteinuria during the hospital stay was used.
11. Oedema. The hospital uses a four-point scale related to location but not intensity of oedema.
    1 = oedema below the malleoli only,
    2 = oedema between the knee and the malleoli,
    3 = oedema between the waist and the knee,
    4 = oedema which involves hands or face regardless of other locations.

    The oedema recorded on admission was used.
12. Birthweight in grams was taken from the medical record.
13. Gestational age as estimated by the admitting physician was taken from the medical record.
14. Gruenwald's Score for retarded fetal growth (Gruenwald, 1966).
15. Fetal sex.

In reviewing the records it soon became clear that information was often incomplete, and that the information which was present had been hastily ascertained and recorded. Nonetheless, the problem with the completeness of these routine data seems to have occurred equally in both the survivors and the stillbirths. For example, when one compared the proportion of stillbirths (19 per cent) and livebirths (14 per cent) who had data missing on age, parity, marriage status or abortion history, one found that the difference was not statistically significant. Thus, the differences between stillbirths and livebirths which have been found are probably genuine. Some of the variables which failed to differentiate between the two groups might have done so if the quality of the data had been better.

### *Analysis*

The 44 stillbirths and 129 livebirths were compared as to the 15 variables mentioned above. Because these variables are highly intercorrelated, an efficient method of analysis was needed to identify predictors in this rather small study. A first step was to identify the factors which seemed independently related to fetal death. A discriminant function analysis was used (Rau, 1952) to find the best *single* discriminator variable then, one by one, each remaining variable was tried in combination with the just selected "single best discriminator". That variable was chosen, which gave the best discrimination when combined with this first selected discriminator. Now these two variables were supplemented, one by one, with each remaining variable, and this process was continued until no new variable added significantly ("t" test at nominal 0·05 level) to the discrimination. Discrimination in all these comparisons was judged by the Mahalanobis $D^2$ (Rau, 1952). Later, birthweight, which was one of the strong discriminators, was replaced by gestational age and RFG for the theoretical reasons already outlined in the companion article (Neutra, 1975). Systolic pressure which had not been included as a candidate variable in the above model was also added.

To verify the results of the discriminant function analysis, a number of cross tabulations have been presented and tested by Chi square for linear trend (Snedecor and Cochran, 1967) and Summary Chi square as appropriate (Mantel and Haenszel, 1959). As a heuristic device a diagram was created to show the relationships of the main predictive variables to fetal death, to each other and to certain other variables of interest. Standardized regression coefficients (Wright, 1921; Land, 1969) were used to show the magnitude and direction of the relationships.

## RESULTS

The discriminant function which was chosen is the sum of the following terms:

| *Variable* | *Coefficient* | "*t*" *Value* |
|---|---|---|
| Unmarried = 1 | 0·935 | 2·27 (167 d.f.) |
| Married = 0 | | |
| Gestational age in weeks | —0·292 | —4·55 (167 d.f.) |
| Systolic blood pressure (mm.Hg) | 0·270 | 3·84 (167 d.f.) |
| Standard deviations from mean weight for age | —0·541 | —2·66 (167 d.f.) |

(Mahalanobis $D^2 = 1\cdot5$)

Seven variables were dropped because they were not associated with fetal death, either when they were taken alone or when they were studied in combination with other factors. These seven variables were:

1. Parity
2. Previous fetal death
3. Barrio Social Class
4. Birthplace
5. Proteinuria
6. Oedema
7. Sex of fetus.

Three variables were related to fetal death when taken alone, but were found unrelated after controlling for other variables: these were:

8. Maternal age
9. Abortion history
10. Diastolic blood pressure.

The eleventh variable, birthweight, was replaced by gestational age and Gruenwald Score for theoretical reasons already mentioned (Neutra, 1975). When a variable is associated with fetal death when taken alone, but is not significantly related when taken in concert with other variables, it means that its original association must have been mediated through one of those other variables. In the case of diastolic pressure, systolic pressure was the variable that accounted for the apparent association. In the case of abortion history and age, however, the interrelationships were not intuitively obvious. Standardized regressions were run between these two variables and the variables which were related to stillbirth. The coefficients significant at the 0·05 level are shown in Figure 1.

Parity has been included in the model since its importance has been stressed in other studies (Zuspan and Ward, 1965). Figure 1 shows that in these data history of abortion and maternal age owed their prediction of fetal death to their association with systolic blood pressure. Similarly, primiparae had a slightly lower risk of fetal death because they were young and tended to have lower blood pressures, because their eclampsia tended to come late in gestation and because they had less RFG.

The following tables corroborate in a more conventional format the findings of the multivariate analysis.

Table I shows that the relation between systolic blood pressure and stillbirth rate was highly significant. Table II shows that the unmarried status carried excess risk of stillbirth just as it had carried excess risk of eclampsia itself. The association was significant in a one-tailed test. The associations between gestational age, RFG and stillbirth have been examined in the companion article (Neutra, 1975). Table III shows the distribution of stillbirths and livebirths as to the discriminant score. This discriminant function based entirely on the four non-therapeutic factors generated groups with a ninefold difference in fetal death rates between the highest and lowest risk category. The Mahalanobis $D^2$ for this formula is 1·5, more than twice the separation attainable using birthweight alone.

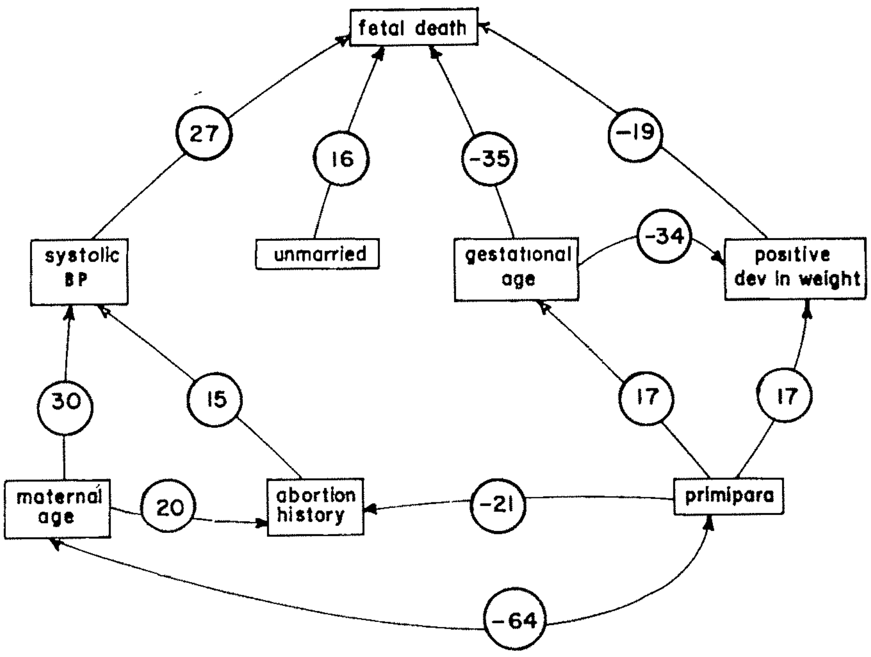

FIG. 1

This diagram summarizes the interrelationships of the variables studied. The encircled numbers are those standardized regression coefficients which were significant at the 0·05 level. They can be interpreted as percentages of a standard deviation. Thus, the "−35" in the arrow leading from gestational age to fetal death means that an increase in one standard deviation of gestational age results in 35 per cent of a standard deviation decrease in the fetal death rate. Maternal age and abortion history are positively correlated with each other and with systolic pressure. Through it they predict fetal death. Primiparity is negatively correlated with abortion history and maternal age, and positively correlated with gestational age and with a positive Gruenwald Score (plump fetuses). Through these variables it protects against fetal death. The unmarried status was weakly correlated with all the other variables and predicts fetal death independently. Systolic blood pressure did not correlate strongly with Gruenwald Score (RFG) or gestational age.

## DISCUSSION

Of the four variables which were found to relate independently to fetal death, we have already discussed the extra risk conveyed by low gestational age and retarded fetal growth (Neutra, 1975). Systolic pressure seemed to measure the severity of the eclamptic episode at least as far as the fetus was concerned. Diastolic pressure was not as strongly related as systolic pressure, a finding similar to that of Peckham (1933). Koch-Weser (1973) has emphasized that systolic blood pressure has strong prognostic value for patients with chronic hypertension. This study shows that systolic pressure has prognostic value for the fetus of eclamptic women as well.

Unmarried women had higher fetal death rates even when controlling for age, parity, systolic pressure, gestational age and RFG. Sutherland (1949) found unmarried women in general to have higher stillbirth rates, even when controlling for parity and age. Since the unmarried status was a predictor of eclampsia in Cali (Neutra, 1973) it is interesting that it appeared again as a predictor of eclamptic fetal death. Unmarried women may have more eclampsia and more fetal death because they use antenatal services differently or because they may share some socio-economic characteristic not detected by the barrio-social class score. A prospective study of toxaemia recently carried out in Cali is now in analysis and may allow the corroboration of these hypotheses. Table II provides some support for a psychosomatic explanation. It shows that the risk of fetal death for spinsters was higher than for women living

TABLE I

*Frequency distribution of stillbirths and survivors by highest systolic blood pressure*

| Systolic blood pressure (mm.Hg.) | Stillborn infants in a category (per cent) | Survivors in a category (per cent) | Stillbirth rate (per cent) |
|---|---|---|---|
| ≤140 | 6·9 | 19·4 | 10·7 |
| 141–159 | 6·9 | 14·7 | 13·6 |
| 160–169 | 18·2 | 14·0 | 30·7 |
| 170–189 | 20·5 | 35·6 | 36·5 |
| 190–209 | 16·0 | 8·5 | 38·8 |
| 210+ | 31·5 | 7·8 | 57·6 |
| Total | 100·0<br>n = 44 | 100·0<br>n = 129 | Overall rate<br>25·4 |

Chi square for linear trend = 12·5 1 d.f. P<0·005.
Chi square deviations = 10·5 4 d.f. P<0·05.

TABLE II

*Stillbirth rate in married, free union and spinster women*

| Marital status | No. of survivors in a category | | No. of stillborn infants in a category | | Stillbirth rate (per cent) | |
|---|---|---|---|---|---|---|
| Married | 65 | | 15 | | 18·8 | |
| Free union | 35 | 64 | 13 | 29 | 27·0 | 31·2 |
| Spinster | 29 | | 16 | | 35·5 | |

Chi square (married *vs.* unmarried) = 3·12 1 d.f.
P = 0·035 (one tailed)

TABLE III

*Percentage distribution of all eclamptic women and eclamptic women with stillbirths as to discriminant score*

| Discriminant score | Proportion of stillbirth mothers in a category (per cent) | Proportion of all the eclamptic mothers in a category (per cent) | Stillbirth rate (per cent) |
|---|---|---|---|
| 15·86–26·50 | 2·5 | 10·4 | 6·3 |
| 26·51–30·20 | 5·0 | 10·4 | 12·5 |
| 30·21–34·50 | 12·5 | 20·1 | 16·1 |
| 34·51–36·86 | 20·0 | 20·1 | 25·8 |
| 36·87–41·43 | 20·0 | 20·1 | 25·8 |
| 41·44–70·55 | 40·0 | 18·9 | 55·2 |
| Total* | n = 40 | n = 154 | Overall rate<br>26·0 |

* Information missing on birthweight and gestational age for some patients.

in free union, a quasi marriage status which was fairly acceptable in the lower classes. This was compatible with the hypothesis that having an illegitimate child is more stressful for a woman who has been abandoned by the child's father than for a woman who has a stable, if not purely legal, relationship. Table IV shows that the extra risk for unmarried women was most marked in women having their first baby. It would seem reasonable that the first illegitimate child would be more emotionally traumatic than the subsequent ones.

Women with a history of abortion had higher stillbirth rates as can be seen from Table V. Further analysis showed abortion history to mediate its effect through its relation with high systolic pressure. Abortion history was a predictor of eclampsia in Cali. Young (1927) in the early part of this century had pointed out what he called "the eclamptic sequence" of abortions and stillbirths preceding an eclamptic pregnancy. The earlier abortions may well be an earlier sign of incipient hypertensive disease.

The effect of age or parity on eclamptic fetal death has been documented in other studies (Zuspan and Ward, 1965; Peckham, 1933; Lopez-Llera *et al.*, 1972), but without controlling explicitly for the close correlation between these two variables or their correlation with other variables. In this study, age, when taken alone, did correlate with fetal death. Parity probably would have if it had been coded as a continuous variable rather than as a dichotomy between primiparity and multiparity. But when taken in the context of other factors, age and parity are seen to owe their apparent association with fetal death to other variables.

Thus Figure 1 showed that primiparae were more likely to develop eclampsia later in gestation and to have less RFG. Since they tend to

TABLE IV

*Stillbirth rates as a function of parity, age and marriage status*

| Parity | Age | Marriage status | Stillbirths | Denominator | Stillbirth rate (per cent) |
|---|---|---|---|---|---|
| Primiparae | ≤20 | Married | 2 | 30 | 6 7 |
| | | Unmarried | 9 | 44 | 20·5 |
| | 21+ | Married | 3 | 12 | 25·0 |
| | | Unmarried | 11 | 20 | 55·0 |
| Multiparae | ≤20 | Married | 2 | 3 | 66·7 |
| | | Unmarried | 1 | 6 | 16·7 |
| | 21+ | Married | 8 | 32 | 25·0 |
| | | Unmarried | 8 | 26 | 30·8 |
| Total | | | n = 44 | n = 173 | Overall rate 25·4 |

TABLE V

*Stillbirth rate as a function of abortion history*

| No history of abortion | History of abortion | Risk relative to no abortion |
|---|---|---|
| 21·2 per cent (151)* | 54·5 per cent (22) | 2·57 |

* Number in parentheses is the denominator on which stillbirth rate was based.

Chi square = 10·3 1 d.f.
P = 0·002.

be younger they are also likely to have lower blood pressure. If we had information about the presence of underlying hypertension we would probably also find that hypertension was less frequent among primiparae. All of these associations tend to protect primiparae from eclamptic fetal death. One might be tempted to say that there are two eclamptic syndromes. One, which occurs in multiparae, has its onset early in pregnancy, is characterized by high blood pressure, marked RFG and high fetal death rates. The other occurs in primiparae, occurs later and with lower blood pressure, less RFG and lower fetal death rates.

The data do indeed indicate some statistical correlations of that kind but they should not be taken to mean that the predictive value of high systolic pressure, RFG, or low gestational age are restricted to multiparae. The data show them working in primiparae as well.

A discriminant score such as that developed in this study may be used to standardize the differences between series of patients from two hospitals who had been subjected to different therapeutic regimens. This could be done by calculating the risk score for each woman in the two series. The two groups of women could then be distributed in categories of risk, as was done in Table III. The results of treatment would then be compared within risk categories. Differences in inherent risk between the two groups could thus be, to some degree, controlled.

## ACKNOWLEDGEMENTS

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# HORMONAL RESPONSES TO A FIRST COURSE OF CLOMIPHENE CITRATE IN WOMEN WITH AMENORRHOEA

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## Summary

The hormonal criteria of a "normal" or "ovulatory" response, and three grades of subnormal anovulatory responses, were defined during a first course of treatment with clomiphene citrate in twenty patients with amenorrhoea for which no organic cause could be found. The hormonal responses were estimated by frequent serum measurements and by the menstrual response. Seven patients had a normal or ovulatory response with a late rise of serum progesterone and subsequent menses. Their serum luteinizing hormone (LH) and oestradiol ($E_2$) responses were greater than in spontaneous menstrual cycles. Five patients gave only partial responses, with menstruation and no prior rise of progesterone. Their serum LH and $E_2$ responses were generally lower than in the normal group but had the same pattern except for an absence of the luteal phase rise in $E_2$. Five patients who did not menstruate showed smaller and more variable responses. The remaining five showed no hormonal responses. The second and third groups had ovulatory responses on subsequent clomiphene courses, whereas no improvement was shown by the last group. The serum $E_2$ responses offered the most sensitive index of the responsiveness to clomiphene. Response values in our normal group suggested that higher progesterone levels need to be reached after clomiphene administration than in spontaneous cycles for ovulation to be inferred. The serum prolactin showed no consistent response during the clomiphene test; three patients with moderately raised basal prolactin levels had "normal" responses.

WHEREAS the response in amenorrhoic women of serum luteinizing hormone (LH), follicle stimulating hormone (FSH) and progesterone to clomiphene administration has been dealt with in several papers (Ross *et al.*, 1970; Marshall and Fraser, 1971; Friedman, 1973; Radwanska and Swyer, 1974), the response of serum oestradiol ($E_2$) and its relation to the other responses has not been reported. Further, although amenorrhoea associated with galactorrhoea has been studied extensively there is only scanty information on serum prolactin in "functional" amenorrhoea or on its response to clomiphene administration.

The present communication therefore reports the serum levels of LH, $E_2$, progesterone and prolactin in a first course of clomiphene in women with functional amenorrhoea. In an attempt to facilitate diagnosis at this early stage and as a guide to further clomiphene treatment we have classified the various responses.

## PATIENTS AND METHODS

### *Patients*

Twenty patients presenting with apparently "functional" amenorrhoea of three months duration or more were chosen for study; patients with clear evidence of a pituitary tumour, other endocrine disorders, ovarian failure or organic gynaecological lesions were excluded.

Further studies of these patients have revealed the following: one patient proved, at laparoscopy, to have the polycystic ovary syndrome (Table I); two had primary amenorrhoea, one due to isolated gonadotrophin deficiency associated with otherwise normal pituitary function, the other a hypothalamic syndrome with associated growth hormone deficiency and short stature; in the other patients, psychological precipitating factors were generally found (Table I), but in five of them the amenorrhoea remained unexplained.

All patients were euthyroid by conventional clinical and biochemical criteria (serum protein bound iodine or thyroxin and/or serum thyroid stimulating hormone). Pelvic examination was normal in all patients except the one with polycystic ovaries.

### *Test procedures*

Clomiphene citrate was given as a dose of 2 mg./kg./day (to the nearest 25 mg.) for seven days except for the patient with the polycystic ovary syndrome who had 50 mg. of clomiphene daily for five days. Blood was taken for two consecutive days when starting treatment and then daily on days 6 to 17, and always between 0800 and 1000 hours. None of the patients had side effects during treatment. In repeated courses, clomiphene was given in an identical way using only measurements of basal body temperature or serum progesterone as indications of ovulation, and pregnancy tests when the menses were delayed.

### *Hormone measurements*

Serum LH was measured by a double antibody radioimmunoassay (Marshall *et al.*, 1972). Using the MRC pituitary standard 69/104 the normal range for the follicular and luteal phases was 2 to 8 U./l. and for the mid-cycle peak 22 to 33 U./l.

### *Serum oestradiol* ($E_2$)

Serum $E_2$ was measured by a highly specific radioimmunoassay using an antiserum raised in

TABLE I

*The aetiology of amenorrhoea in patients grouped according to their response to clomiphene*

| Responses to clomiphene | Diagnosis | No. |
|---|---|---|
| "Normal" (ovulation and menses) | Post pill amenorrhoea | 3 |
| | Anorexia nervosa | 2 |
| | Polycystic ovary syndrome | 1 |
| | Unknown | 1 |
| Partial (menses only, no ovulation) | Anorexia nervosa | 2 |
| | Unknown | 3 |
| Minimal (hormonal response but no ovulation or menses) | Anorexia nervosa | 3 |
| | Depressive episode | 1 |
| | Unknown | 1 |
| Nil | Isolated gonadotrophin deficiency* | 1 |
| | Hypogonadotrophic amenorrhoea† | 1 |
| | Hypothalamic amenorrhoea* | 1 |

* Primary amenorrhoea.

† Secondary amenorrhoea without evidence of pituitary tumour.

rabbits against oestradiol-6-oxime-bovine serum albumin. Tritiated $E_2$ was employed both as the label in the assay itself, and as an internal standard added to samples in order to assess the recovery in the diethyl ether extraction step. Dextran coated charcoal was used to separate bound and free $E_2$. The intra-assay and inter-assay coefficients of variation were 8·6 per cent and 12·2 per cent respectively.

The normal range of the first ten days of the follicular phase was 27 to 130 pg./ml., 51 to 276 pg./ml. for the midcycle ten days and 59 to 160 pg./ml. for the last ten days of the luteal phase.

*Serum progesterone*

Serum progesterone was measured by radioimmunoassay using an antiserum raised in rabbits against progesterone-11-glutamate-bovine serum albumin. Petroleum ether (boiling range 40 to 45 °C.) was employed to extract the serum samples but in other respects the assay was similar to the $E_2$ assay which is outlined above. The normal range of the follicular phase was less than 0·6 ng./ml., and 1·6 to 18·5 ng./ml. for the luteal phase. Intra-assay and inter-assay coefficients of variation were 9 per cent and 15·6 per cent respectively.

*Serum prolactin*

Serum prolactin was measured by double antibody radioimmunoassay using antiserum raised against human prolactin. Human prolactin for labelling was supplied by the National Institutes of Health, Bethesda, Maryland (VLs No. 1). Our normal female range was 2 to 25$\mu$g./l. Intra-assay and inter-assay coefficients of variation were 14 per cent and 21 per cent respectively. Human prolactin 72/4/9 was used as a standard.

## RESULTS

*Types of responses to clomiphene*

After reviewing the hormonal and menstrual responses we have set aside seven as normal responses since they were associated with both menstruation and ovulation, the latter being inferred when the serum progesterone rose to over 2·0 ng./ml. on the first day after the LH peak or higher values on subsequent days (Table II, Fig. 1). As shown in Table III, the other responses are then readily grouped into one of three types of subnormal responses, namely "partial" (menses without ovulation), "minimal" (some hormonal response but neither ovulation nor menses) and "nil" (no response at all).

*The "normal" response.* The hormonal responses of a representative patient are shown in Figure 1, while Table II presents for all seven such patients the main features of these "normal" responses to clomiphene. In this group menses always occurred by the 26th day after starting clomiphene. From Table II it may be noted that basal values and responses in this group tended to be higher than in the subnormal groups. $E_2$ and progesterone levels reached in the responses were, with one exception, higher than the corresponding ones in spontaneous cycles and they did not, with the same exception, overlap with the responses of the subnormal groups.

*The "partial" response.* Five patients menstruated after clomiphene administration but did not ovulate as judged by their serum progesterone level remaining 2 ng./ml. or less (Table III). All these patients had a second LH peak but as for other responses in this group they were generally smaller than those in the normal group. The menstrual response was irregular and tended to be delayed. All patients in this group later gave "ovulatory" responses in repeated clomiphene courses and pregnancies eventually occurred.

*The "minimal" response.* The five patients in this group had generally low basal serum LH levels and no secondary LH peak together with a negligible progesterone rise. However, they all responded with a considerable rise in serum $E_2$ although these elevations were either of short duration during the clomiphene administration or protracted. Four patients in this group are known to have had normal responses on further clomiphene administration.

*The "nil" response.* Here basal serum levels of both LH and $E_2$ were subnormal and no responses to clomiphene were detected. Two of the three patients had primary amenorrhoea of pituitary-hypothalamic origin (Table I).

*Serum prolactin levels*

In the group with normal responses, four patients had initial prolactin levels within the

TABLE II

*Data about patients who responded to clomiphene citrate by ovulation and menstruation*

| No. | Diagnosis | Age | Serum LH (U./l.) | | | | Serum $E_2$ (pg./ml.) | | Serum progesterone | | Day of onset of menses | Duration of luteal phase (days) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Basal | Primary peak | Secondary peak | Day of secondary peak | Basal | Peak | Highest levels measured (ng./ml.) | Days after LH peak | | |
| 1 | Post pill amenorrhoea | 26 | 4·1 | 6·2 | 26 | 12 | 30 | 726 | 23·5 (7·6)* | 5 | 25 | 13 |
| 2 | Post pill amenorrhoea | 25 | 3·8 | 15·5 | 55·0 | 14 | 27 | 1306 | 16 (4·3) | 3 | 26 | 12 |
| 3 | Post pill amenorrhoea | 23 | 3·0 | – | 12·5 | 11 | 25 | 1480 | 4·9 (4·3) | 4 | 20 | 9 |
| 4 | Anorexia nervosa | 24 | 4·5 | 12·5 | 32 | 12 | 27 | 1045 | 8·0 (4·3) | 4 | 19 | 7 |
| 5 | Anorexia nervosa | 18 | 3·3 | 7·6 | 16 | 16 | 20 | 3784 | 3·9 (1·5) | 1 | 20 | 4 |
| 6 | Polycystic ovary syndrome | 25 | 3·4 | 7·2 | 6·0 | 12 | 6·2 | 676 | 10·8 (4·3) | 3 | 21 | 9 |
| 7 | Unknown | 25 | 6·4 | 10·5 | 30 | 12 | 23 | 228 | 4·5 (4·3) | 2 | 23 | 11 |

* Mean normal plasma progesterone for the given day after the LH peak in spontaneous ovulatory cycles (Ross *et al.*, 1970) are shown in parentheses.

TABLE III

*Data about response to a clomiphene citrate test in 20 patients with "functional" amenorrhoea. The dose of clomiphene citrate used was 2 mg./kg./day for 7 days in all but one patient. The latter had polycystic ovaries and was only given 50 mg. of clomiphene citrate daily for 5 days.*

| Type of response to clomiphene | No. | Serum levels (median and range) | | | | | | | Days to secondary peak of LH | Duration of luteal phase (days) | Day of onset of menstruation |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | LH (U./litre) | | | Oestradiol (pg./ml.) | | Highest level of progesterone measured (ng./ml.) | Basal prolactin level (µg./litre) | | | |
| | | Basal | Primary | Secondary | Basal | Peak | | | | | |
| Normal (ovulation and menses) | 7 | 3·9<br>(3·0–6·4) | 10·5<br>(6·2–15·5) | 26<br>(6·0–55·0) | 25<br>(6·2–30·0) | 726<br>(228–3784) | 8·0<br>(3·9–23·5) | 7·2<br>(4·7–80) | 12<br>(11–16) | 9<br>(4–13) | 21<br>(19–26) |
| Partial (menses only, no ovulation) | 5 | 3·3<br>(0·55·0) | 6·2<br>(5·0–8·4) | 12<br>(5·7–14·0) | 20<br>(11–80) | 267<br>(139–435) | 1·5<br>(0·9–2·0) | 6·8<br>(5·8–22) | 13<br>(10–15) | – | 27<br>(19–47) |
| Minimal (hormonal response but no ovulation or menses) | 5 | 1·5<br>(1·0–3·2) | 6·2<br>(3·8–12·0) | – | 26<br>(18–36) | 375<br>(166 455) | <1·2 | 5·4<br>(3·8–6·2) | – | – | – |
| Nil | 3 | 1·0 | 1·0 | – | 6·2<br>(5·6–15·0) | – | <0·6 | 3·4<br>(<2–9·6) | – | – | – |

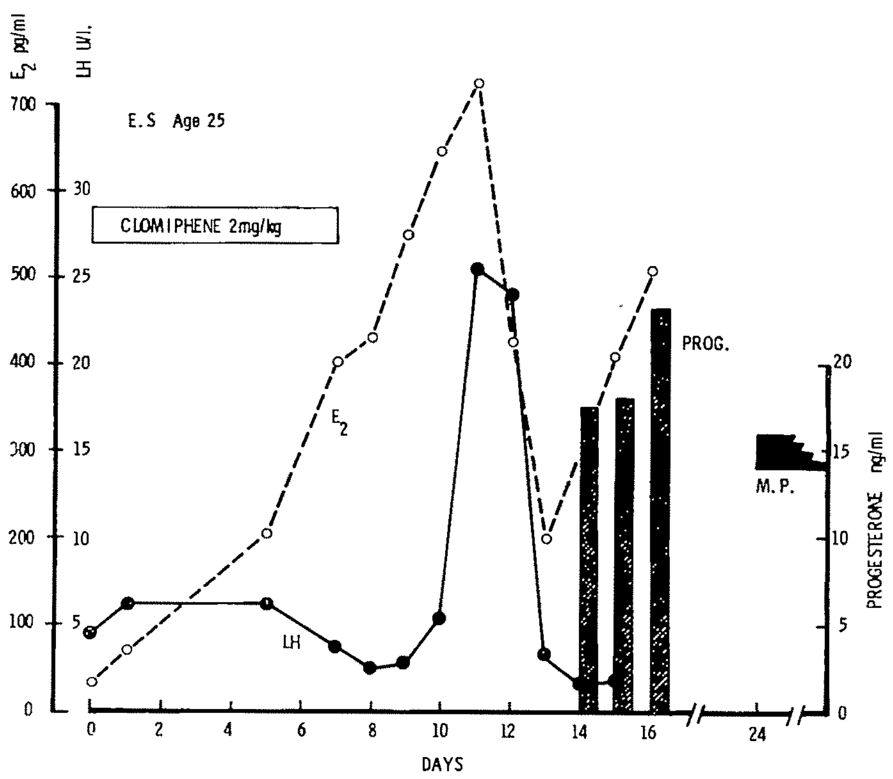

FIG. 1

Hormone responses seen in an amenorrhoeic subject who had a "normal" response to a standard clomiphene test. M.P. = menstrual period; PROG. = progesterone.

normal range and three had had moderately elevated levels (47 to 80 μg./l.; Table III). The levels were followed throughout the test in one with normal basal levels and two with high levels. While the higher levels fluctuated there was no trend of change during the test.

Among the patients who showed subnormal responses to clomiphene, none had initially high prolactin levels. In three of these, followed throughout the cycle, no changes were noted.

## DISCUSSION

The ease of administration, relatively minor side effects and efficiency in restoring ovulation and menses often make clomiphene citrate the first treatment in "functional" amenorrhoea associated with infertility. Eighty per cent of such patients can be expected to ovulate and approximately 50 per cent become pregnant (Kase, 1973). The findings in our small group of patients seem to confirm these figures.

The hormonal responses to a first course of clomiphene can give clues to diagnosis. Measurements of total hormone excretion in urine, though often used in the past, may not satisfactorily reflect the effective levels of the hormones at the target cells. This applies particularly to the sex steroids some of which are secreted into the blood stream both free and as conjugates which are then cleared at a different rate into the urine (Baulieu and Robel, 1970). We therefore measured the unconjugated $E_2$ in serum during and after standard courses of clomiphene.

Although the basal serum $E_2$ levels showed tenfold differences between comparable individuals serial serum $E_2$ measurements provided the most sensitive index of the response to clomiphene. Thus when the serum $E_2$ levels rose to values above 460 pg./ml. ovulation always followed. Further, the $E_2$ responses allowed us to define in the first clomiphene course a group of patients that neither ovulated nor menstruated ("minimal" response) but who were still able to respond normally as 80 per cent of them ovulated later after repeated courses of clomiphene. The finding of considerably higher peak levels of $E_2$ in our normal group than in spontaneous cycles is in agreement with the results obtained by Robertson *et al.* (1971).

The striking finding of four short luteal phases in seven of the normal response group also supports the claim that in many cases clomiphene needs to be supplemented by some luteotrophic agent or progesterone itself to improve prospects of pregnancy (Kistner, 1966; Rabau *et al.*, 1968; Greenblatt *et al.*, 1971; Keller, 1972; Insler *et al.*, 1973; Jones, 1973). Repeated clomiphene-induced cycles have to be studied further, however, to establish the overall incidence of this phenomenon.

While the highest progesterone level measured in the groups of patients with subnormal responses was 2·0 ng./ml., the lowest progesterone level measured in the normal response group during the luteal phase was 1·9 ng./ml. on the first day after the LH peak. We have, therefore, used the value of 2·0 μg./ml. on the first day, combined with later values if obtained, as the dividing point between the normal and subnormal responses. Our normal progesterone values (1·6 to 18·5 ng./ml.) are based on studies throughout four normal luteal cycles and 14 samples from different individuals during the luteal phase; they have a similar range (approximately 1·0 to 15 ng./ml.) to those given by Ross *et al.* (1970). We have therefore given their mean values for reference in Table II and it will be seen that our values for the corresponding day are always higher. This is in agreement with Robertson *et al.* (1971) who reported higher levels of serum progesterone after clomiphene administration than those seen in spontaneous cycles. Although this may be related to the clomiphene dose given, our results would suggest that in the normal clomiphene response serum progesterone levels higher than those in spontaneous cycles must occur before ovulation can be inferred. This in turn might have some bearing on the incidence of luteal insufficiency or short luteal phase found in our study and that of Van Hall and Mastboom (1969).

Basal levels of the hormones measured gave little information about the potential response to clomiphene although no response is likely to follow very low levels of both serum LH and $E_2$. No correlation was found between duration of amenorrhoea and hormonal responses.

It is perhaps of interest that all three patients with elevated prolactin levels showed a normal response to clomiphene, and the drug did not appear to have any effect on prolactin levels.

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# ENDOMETRIOID TUMOURS OF THE OVARY

BY

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AND

C. N. HUDSON

*For the*
*Association of Obstetricians and Gynaecologists and Regional Histopathologists Group of the North East Metropolitan Region*

## Summary

From 291 cases of malignant disease of the ovary in the North East Metropolitan Region of London, 250 have been classified as of epithelial origin. Of these, 44 have been designated as endometrioid carcinoma. The recognition and features of endometrioid ovarian carcinoma are discussed.

THE common epithelial tumours of the ovary arise from the surface or "germinal" epithelium. It is postulated that differentiation occurs into the varieties of epithelium seen in the Müllerian tract. Mucus secreting (mucinous) tumours bear an obvious resemblance to endocervical epithelium and serous papillary tumours may be equated with the epithelium lining the oviduct (Fox, 1970). The recognition, however, of a group of tumours representing differentiation into endometrial epithelium is less easy. Sampson (1925) described a variety of malignant ovarian tumour which closely resembles the histological pattern of carcinoma of the endometrium. Although others have since recognized this group, it was not until 1961 that the International Federation (FIGO) specified them as a separate entity in their ovarian tumour classification, the designation "endometrioid" being employed. A detailed report on 78 cases was made by Czernobilsky *et al.* (1970*b*).

The problems of recognition of this endometrioid group are not purely those of unfamiliarity. There is lack of agreement on the criteria for diagnosis (Gray and Barnes, 1967). These two factors have obviously detracted from the value of many contributions on ovarian cancer, as they have made it well nigh impossible to compare series prepared against the background of different pathologists' opinions. This presentation is concerned with the recognition of endometrioid cancer in a group of 250 patients with malignant epithelial tumours of the ovary, and attempts to clarify the salient features of this group.

## METHODS

Since September 1972 a tumour registry for malignant disease of the ovary has been maintained in the North East Metropolitan Region of London, an area of some 1650 square miles with a population estimated in 1973 at 3 389 000. This area includes a quarter of the Greater London area and more rural districts of Hertfordshire and Essex. All patients diagnosed have been registered including those "imported" for treatment. The histopathology of all tumours notified to the study was examined independently by the two investigators without reference to the local pathologist. When there was disagreement or uncertainty the slides were referred to a panel of interested pathologists.*

Haematoxylin and eosin staining was used for routine examination. The special stains used

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* Reference Panel of Pathologists:

| | |
|---|---|
| Dr. C. Raeburn | Whipps Cross Hospital |
| Dr. A. G. Stansfeld | St. Bartholomew's Hospital |
| Dr. W. J. Harrison | North Middlesex Hospital |
| Dr. J. Landells | The London Hospital |
| Dr. J. O. W. Beilby | Hospital for Women. |

were Periodic Acid Schiff, Alcian Blue and Best's Carmine. The presentation data from the first 250 patients were processed for analysis.

## FINDINGS

The relative proportions of the common epithelial tumours are illustrated in Figure 1. The 44 cases that we have classed as endometrioid carcinoma of the ovary are further subclassified in Figure 2. The distribution by FIGO stage and by counting mitotic figures (Dyson *et al.*, 1971) is shown in Table I.

TABLE I

*Distribution of 44 endometrioid tumours by FIGO staging and mitosis count*

| FIGO stage | | | | Mitosis count (per high-power field) | | |
|---|---|---|---|---|---|---|
| I | II | III | IV | 0–2 | 2–4 | more than 4 |
| 12 | 6 | 15 | 11 | 13 | 30 | 1 |

The classical endometrioid carcinoma (Fig. 3) and the adeno-acanthoma (Fig. 4) are easy to identify, whereas the group of "hybrid" tumours, which show serous papillary, mucinous or clear cell elements, and the poorly differentiated tumours are more difficult. The eleven tumours showing mixed epithelial elements included one which showed all three types of epithelium (Fig. 5). Three tumours of this "hybrid" group also showed squamous metaplasia. These were in many cases seen by members of the reference panel before the decision to include them in the endometrioid category was taken.

### *Endometrial histology*

Endometrium was available for histological examination in 34 patients but in one of these from curettings only. Endometrial carcinoma was present in 9 patients (6 adeno-acanthoma, 1 papillary, 1 clear cell (Fig. 6), 1 undifferentiated). There were three endometrioid tumours associated with cystic endometrial hyperplasia. Two of these patients were premenopausal and the other postmenopausal but with no postmenopausal bleeding. A further two ovarian endometrioid tumours were associated with uterine endometrial polyps but the remainder were unremarkable.

## DISCUSSION

The endometrioid group of cancers embrace a variety of tumours of epithelial origin which do not bear a clear resemblance to tubal or endocervical epithelium. These may include a number of "clear cell" carcinoma cases which need to be distinguished from clear cell (mesonephroid) carcinoma which are now classified separately (Serov *et al.*, 1973), but for which a Müllerian histogenesis has been postulated (Scully and Barlow, 1967). At the present time mesonephroid carcinoma is grouped separately in the WHO classification.

Long and Taylor (1964), following a suggestion by Santesson (1961), defined certain histological characteristics for endometrioid tumours. The main criteria for diagnosis are those of well-recognized cancer of the endometrium and may be summarized as follows:

(1) Tumours should have a tubular or acinar arrangement with a single or multiple layer of cells in the better differentiated grades. In the poorly differentiated tumours, the inherent glandular nature should be recognizable in part of the tumour.

(2) As the underlying stroma is different, the "back to back" glandular appearance characteristics of endometrial cancer will not be so apparent in endometrioid ovarian tumours.

(3) If the tumours are papillary, the papillae have a blunt appearance in contrast to the finer branching of the common serous papilliferous tumours.

(4) Squamous metaplasia (adeno-acanthoma) is very characteristic of this group, and, when present, is almost pathognomonic.

(5) Glycoproteins and nonspecific mucins may be present, particularly in the apical region of the epithelial cells. Extracellular mucin may be present in gland lumina.

Some or all of the above features were predominant in the cases labelled as endometrioid in the present series. Many endometrioid cancers have an obvious papillary appearance. Together with the mucus secretion mentioned above, this fact accounts for overlap at both ends of the histological spectrum. In the poorly differentiated tumours, it is particularly difficult to be confident about the correct application of the label endometrioid. There is thus a subjective

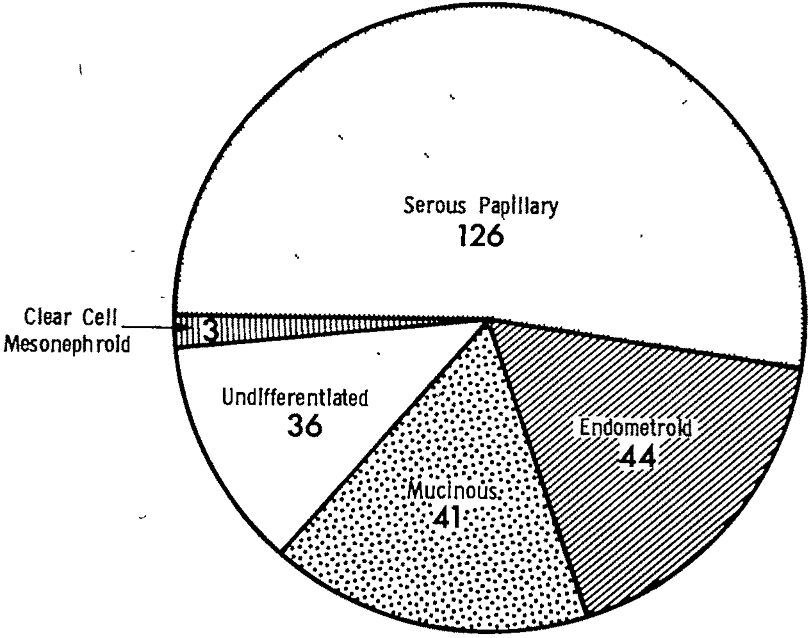

FIG. 1
Distribution of tumour type in 250 patients according to the WHO classification. The numbers indicate the size of each group.

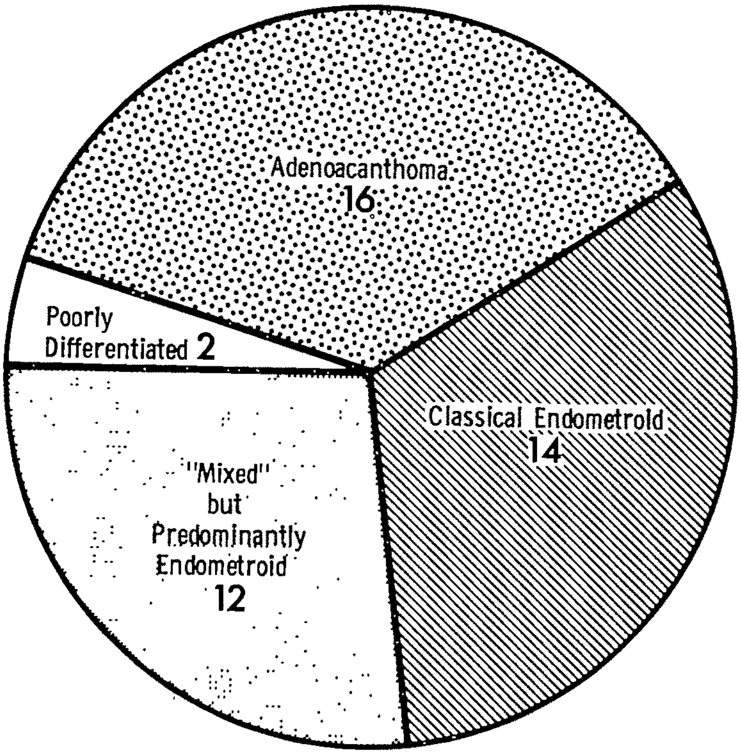

FIG. 2
Subdivision of the 44 endometrioid tumours. The numbers indicate the size of each group.

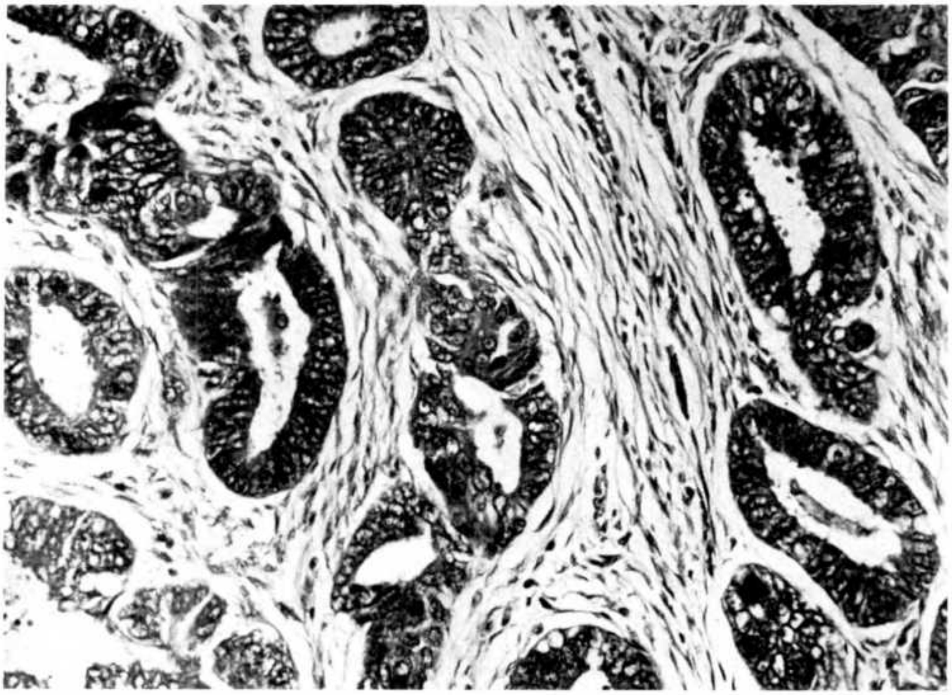

FIG. 3

A well-differentiated endometrioid carcinoma. (Haematoxylin and eosin ×200).

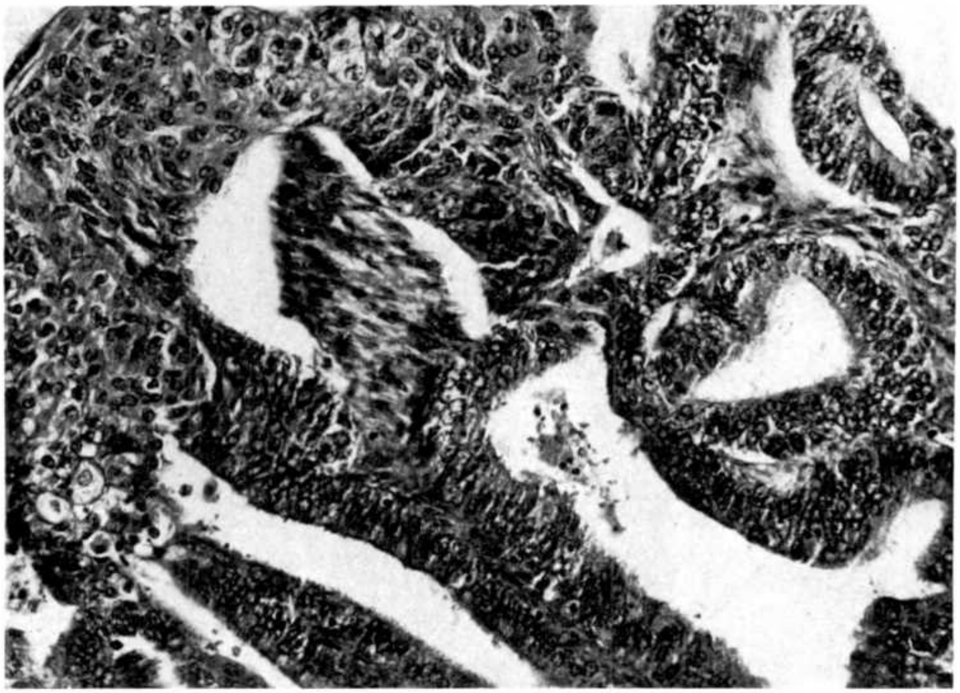

FIG. 4

Squamous metaplasia in an endometrioid carcinoma making a diagnosis of adenoacanthoma. (Haematoxylin and eosin ×200).

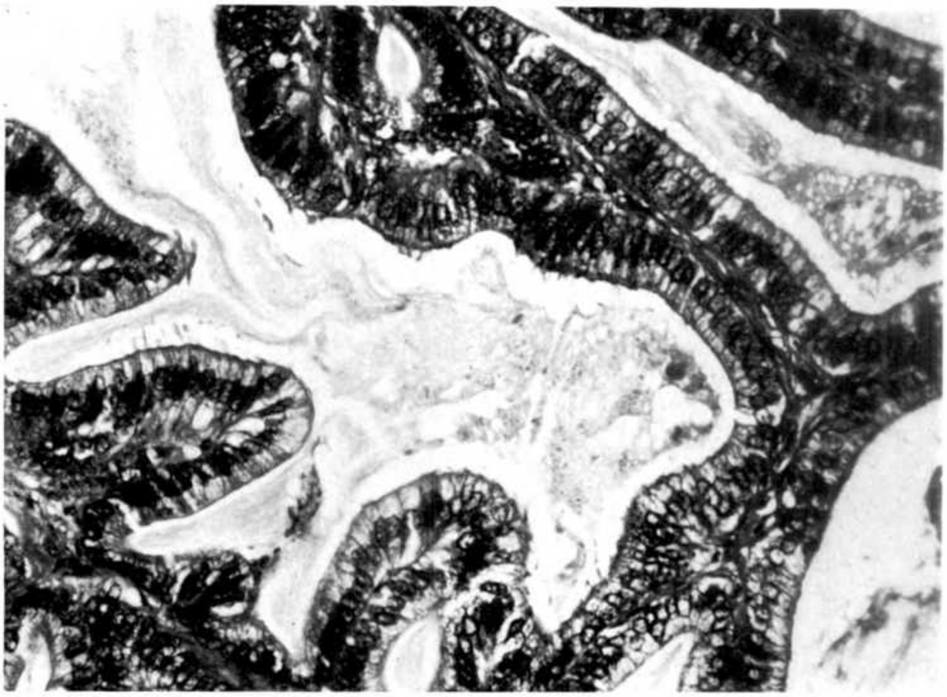

FIG. 5

An area of mucus secreting epithelium selected from a "hybrid" endometrioid tumour. (Haematoxylin and eosin ×200).

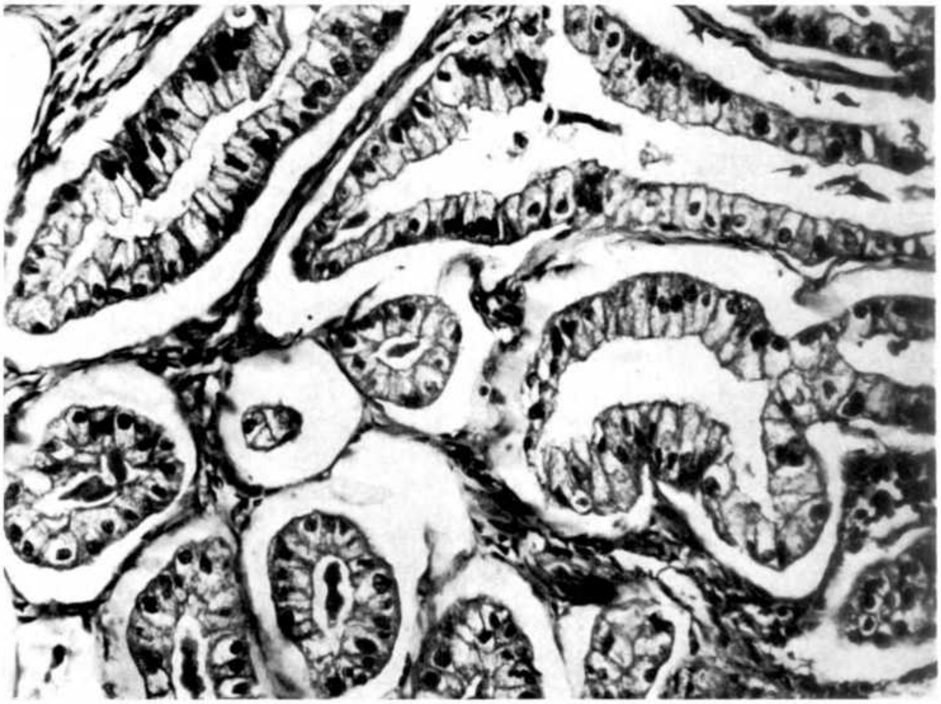

FIG. 6

"Clear cell" appearance seen in an endometrial carcinoma. The ovarian histology was similar. (Haematoxylin and eosin ×200).

element of selection in the identification of this group so that it may contain a disproportionate number of well-differentiated cases. This artefact may be responsible for an allegedly better prognosis (Schiller and Kirol, 1966).

The well-recognized tendency to pleomorphism in ovarian tumours has helped to mask the existence of "hybrid" tumours in which two, or occasionally three, types of epithelial tumour tissue are present. Only when a determined attempt is made to allocate all ovarian tumours to the groups laid down in the FIGO classification is such a residuum apparent.

### *Clear cell tumours*

One example of pleomorphism found in both serous and endometrioid tumours is the clear cell variant (Kurman and Craig, 1972). At present these cases are classified separately if the clear cell element is predominant. Czernobilsky *et al.* (1970*a*) support the view that the majority of these tumours are of Müllerian origin and mostly endometrioid at that. Distinction must further be made from the yolk sac tumour of adolescents (Beilby and Todd, 1974). This tumour is also called endodermal sinus tumour (Teilum, 1959) and was originally included in Schiller's description of a mesonephroma (Schiller, 1939). It is now considered to be a teratoma of extra-embryonic origin. The clear cell (mesonephroid) tumours generally occur in an older age group, and have characteristically a microcystic structure, clear cell areas and, in places, a fine papillary structure with typical nuclear hobnailing (Hayes, 1972).

One of the factors which support the association of clear cell carcinoma with more typical endometrioid tumours is the occurrence of a similar appearance in uterine carcinoma. Indeed classical mesonephric epithelium has been described in an endometrial polyp (Fechner, 1968). One of our patients showed a clear cell carcinoma of both endometrium and the ovary (See Fig. 6).

### *Association with carcinoma of the endometrium*

In a series of cases of endometrioid cancer reported by Kottmeier (1965) approximately one-third were associated with a malignant tumour of the endometrium. The possibility of this association is of practical as well as theoretical importance as it remains the major indication for hysterectomy in carcinoma of the ovary. The actual incidence is difficult to establish as pathologists will differ in whether they will report the ovarian tumour as primary and the uterine as secondary or vice versa. We have preferred not to attempt any such distinction, particularly as histological differences sometimes suggest that the tumours are separate primaries arising, perhaps, in a common Müllerian field. If the presence of tumour in both uterus and ovary always implies metastatic disease, the prognosis should be correspondingly poor. This particular point is being closely examined in the current study. So far we have not found a carcinoma of the endometrium associated with any ovarian tumour other than endometrioid, or one of the granulosa/theca cell series. It is of interest that one patient had a granulosa cell carcinoma in one ovary and an endometrioid cancer in the other, although the endometrium was not malignant or hyperplastic. There has been one case of a mucus-secreting carcinoma of the endocervix associated with a mucinous tumour of the ovary, which may represent a comparable situation.

### *Endometriosis*

Sampson (1925) described malignant change in pelvic endometriosis and laid down strict criteria for making the diagnosis. It has been seen in extrapelvic endometriomata, even in an operation scar (Howkins, 1956). Such malignant change normally has an endometrioid pattern and it often shows squamous metaplasia (Novak and Woodruff, 1963). We found evidence of pelvic endometriosis in 11 of our patients, including one in whom there was probable malignant change in adenomyosis interna in the anterior uterine wall. In six the evidence of endometriosis was found in the malignant ovary although a transition was only demonstrated in one.

There is, however, no theoretical reason to postulate an origin in endometriosis for all malignant endometrioid ovarian tumours. The benign counterpart of the latter should be a cyst lined with epithelial cells similar to endometrium but without endometrial stroma. These may normally be classed as adenofibroma or cystadenofibroma, and Langley (1973) has described squamous metaplasia in such a tumour.

*Therapeutic considerations*

The potential association with endometrial cancer suggests that curettage should be performed if, for some reason, the uterus is not removed during any operation for malignant ovarian disease. The management of the second ovary also needs careful consideration. The propensity for bilateral involvement in the serous papilliferous tumours is well recognized but is not so obvious in the endometrioid group. Nevertheless caution should be exercised over conservation, and if any apparently normal second ovary is to be retained in a young woman it should be biopsied. Endometrioid tumours are, however, not common under the age of 40. The mean age of our group was 55·5, while the mean age of all 250 patients was 56·7. The difference between the two age groups using the unpaired "t" test was not significant ($P>0 \cdot 05$). Nevertheless, 21 out of 44 patients were recorded as being premenopausal and this included four out of nine patients with associated endometrial cancer, none of whom had had abnormal uterine bleeding other than menorrhagia.

It is possible that these tumours extend locally without at first giving rise to multiple or distant metastases. Hudson and Dendy (1974) found eight endometrioid out of 23 malignant tumours suitable for extended local operations. By the same token residual growth after incomplete surgery is likely to be confined to the pelvis and thus considered suitable for radiotherapy. The efficacy of radiotherapy in such cases has yet to be convincingly demonstrated although its use combined with surgery in uterine endometrial cancer is well established. On theoretical grounds, one might expect the benefits of progestogen therapy claimed for ovarian cancer (Ward, 1972) to be particularly applicable to this group.

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# REPLACEMENT HORMONE THERAPY IN GONADAL DYSGENESIS

BY

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## Summary

Curettage carried out during the third week of treatment with ethinyl oestradiol in 14 patients with gonadal dysgenesis showed cystic glandular hyperplasia in seven patients with some hyperplastic changes in one other. One of these seven patients developed a large endometrial polyp and in one atypical changes were found in the hyperplastic endometrium. In seven patients treated by an oestrogen-progestogen combination normal secretory endometrium was seen in five, proliferative change only in one (thought to be due to incorrect timing of the curettage) and a small proliferative polyp in association with secretory endometrium elsewhere in the uterus in the seventh. In view of these changes and report of adenocarcinoma of the endometrium in patients with gonadal dysgenesis treated with oestrogens alone it is suggested that a regime of low dose oestrogen therapy for 21 days accompanied by a progestogen on days 15 to 21 is preferable to oestrogens alone.

REPORTS of the development of adenocarcinoma of the uterine body in patients with gonadal dysgenesis receiving oestrogen therapy (Dowsett, 1963; Canlorbe *et al.*, 1967; Scott, 1967; Cutler *et al.*, 1972 and Wilkinson *et al.*, 1973) raise the question of the safety of treatment of this kind. Most of these reports, and others linking oestrogen therapy with uterine cancer (Gusberg and Hall, 1961; Vass, 1949; Jensen and Østergaard, 1954; Wallach and Henneman, 1959), have concerned relatively high dosage given for prolonged periods of time. Patients with gonadal dysgenesis do require oestrogens for many years, and although high doses are not necessary the safety of low doses given over many years remains in doubt. One of us (CJD) has customarily prescribed ethinyl oestradiol in doses of 0·02 mg. daily for three consecutive weeks in every month for women with gonadal dysgenesis and so it seemed wise to ascertain the character of the endometrium in these and other patients under our care in order to discover whether a raised cancer risk was involved.

## THE STUDY

Endometrium was obtained by curettage in 14 patients with gonadal dysgenesis who had been receiving intermittent oestrogen therapy for three consecutive weeks in every four weeks for two years or more. Endometrial samples were also obtained from seven further patients with gonadal dysgenesis who had been treated with oestrogens alone for two weeks and oestrogen combined with a progestogen during the third week of treatment. All curettages were performed during the third week of therapy.

## RESULTS

The characteristics of the patients in the study and the type of endometrium present are set out in Table I. It is evident that hyperplasia of the

TABLE I

*Clinical and histological information about patients studied.*

| Patient No. | Age | Karyotype | Preparation and dosage | Duration of treatment | Endometrial histology |
|---|---|---|---|---|---|
| 1 | 18 | 45X | EO 0 1 mg.<br>Day 1–21 | 4½ years | Large polyp<br>CGH |
| 2 | 16 | 45X | EO 0·02 mg.<br>Day 1–21 | 4 years | Proliferative pattern |
| 3 | 27 | 45X | EO 0·02 mg.<br>Day 1–21 | 4½ years | CGH with atypical change |
| 4 | 19 | 45X | EO 0·02 mg.<br>Day 1–21 | 2½ years | Proliferative pattern |
| 5 | 27 | 45X | EO 0·02 mg.<br>Day 1–21 | 10 years | CGH |
| 6 | 27 | 47XXX | EO 0·02 mg.<br>Day 1–21 | 3 years | Proliferative pattern<br>Slight hyperplasia |
| 7 | 18 | 45X/46XXqi | EO 0–02 mg.<br>Day 1–21 | 2 years | Proliferative pattern |
| 8 | 20 | 45X/46XX | EO 0 02 mg.<br>Day 1–21 | 3 years | Proliferative pattern |
| 9 | 22 | 45X/46XY | EO 0–02 mg.<br>Day 1–21 | 3 years | CGH |
| 10 | 19 | ? | EO 0·02 mg.<br>Day 1–21 | 4 years | CGH |
| 11 | 22 | 46XX | EO 0·04 mg.<br>Day 1–21 | 3 years | CGH |
| 12 | 25 | 46XX | EO 0·02 mg.<br>Day 1–21 | 2 years | Proliferative pattern |
| 13 | 23 | 46XX | EO 0·2 mg.<br>Day 1–21 | 4 years | CHG |
| 14 | 21 | 46XY | EO 0·02 mg.<br>Day 1–21 | 2½ years | Proliferative pattern |
| 15 | 23 | 45X/46XY | EO 0·05 mg.<br>Day 1–21<br>Norethisterone 5 mg<br>Day 15–21 | 6 years | Polyp+Secretory endometrium |
| 16 | 18 | 45X/46XX | EO 0 04 mg.<br>Day 1–21<br>Norethisterone 5 mg.<br>Day 15–21 | 3 years | Early secretory pattern |
| 17 | 20 | 45X/46XY | EO 0·1 mg.<br>Day 1–21<br>Norethisterone 5 mg.<br>Day 15–21 | 5 years | Early secretory pattern |

TABLE I—*continued*

| Patient No. | Age | Karyotype | Preparation and dosage | Duration of treatment | Endometrial histology |
|---|---|---|---|---|---|
| 18 | 26 | 46XY | EO 0·1 mg.<br>Day 1–21<br>Megestrol Acetate 1 mg.<br>Day 15–21 | 3 years | Secretory pattern |
| 19 | 28 | 46XY | EO 0·01 mg.<br>Day 1·21<br>Norethisterone 5 mg.<br>Day 15–21 | 4½ years | Early secretory pattern |
| 20 | 23 | 46XX | Premarin 1·25 mg.<br>Day 1–21<br>Medroxyprogesterone<br>5 mg. Day 15–21 | 3 years | Secretory pattern |
| 21 | 40 | 46XX | EO 0·02 mg.<br>Day 1–21<br>Norethisterone 5 mg.<br>Day 15–21 | 4 years | Proliferative pattern |

EO = Ethinyl Oestradiol.
CGH = Cystic glandular hyperplasia.

endometrium is likely with unopposed oestrogen therapy, even in low doses. Seven of the 14 patients treated in this way showed cystic glandular hyperplasia and one other showed some evidence of hyperplasia in an endometrium which, as a whole, did not possess the complete characteristics of cystic glandular hyperplasia. By contrast, five of the seven patients who had received an oestrogen which during the third week of administration was combined with a progestogen showed endometrium with normal secretory or early secretory changes. In one case the endometrium was proliferative but this was thought due to the curettage being carried out at the beginning of the third week instead of later. One patient had a small endometrial polyp showing a proliferative pattern although secretory changes were present in the remaining endometrium.

Special mention must be made of four patients (Nos. 1, 3, 5 and 15). Patient No. 1 had been on a very high dose of ethinyl oestradiol for 4½ years (0·1 mg. daily for three weeks in every month prescribed elsewhere) and had marked endometrial hyperplasia with the formation of an endometrial polyp the size of a thumb which was protruding through the cervix; this case has been reported elsewhere (de Koos, 1974). Patient No. 3, however, had cystic glandular hyperplasia with atypical cellular activity despite being on a low dose of oestrogen (ethinyl oestradiol 0·02 mg. daily) for only four years. Patient No. 5 who had been on oestrogen therapy for the longest time of ten years had suffered from some irregular bleeding with breakthrough over the six months before curettage was performed and "cystic glandular hyperplasia" found. Patient No. 15, who had developed an endometrial polyp on combined therapy, had been receiving 0·05 mg. of ethinyl oestradiol daily and had been on treatment for the second longest time of six years.

## DISCUSSION

These results are not conclusive but they support the view that oestrogen therapy alone in patients with gonadal dysgenesis is likely to provoke endometrial hyperplasia; this hyperplasia may give rise to irregular vaginal bleeding and may proceed to polyp formation; ultimately the development of malignant change must be considered a real risk. The likelihood that the development of adenocarcinoma of the endometrium in the patients with gonadal dysgenesis treated with diethylstilboestrol was due to the therapy is supported by several facts. The average age of these patients at the time of diagnosis was 30·9 years compared with the mean age of 55 years for adenocarcinoma in genetically-normal individuals. Moreover, only two examples of spontaneous endometrial carcinoma in untreated patients with dysgenetic gonads have been described (Lewis *et al.*, 1963: Gray *et al*, 1970). Other papers in the literature have linked unopposed oestrogen therapy with endometrial adenocarcinoma (Gusberg and Hall, 1961; Vass, 1949; Jensen and Østergaard, 1954; Wallach and Henneman, 1959), whilst progressive changes from cystic glandular hyperplasia to adenomatous hyperplasia and finally to adenocarcinoma (Gusberg *et al.*, 1954; Meissner *et al.*, 1957; Fremont-Smith *et al.*, 1946; Gusberg, 1947; Hertig and Sommers, 1949; Hertig *et al.*, 1949) have been demonstrated in the endometrium of patients receiving prolonged oestrogen therapy.

In the patients referred to here with gonadal dysgenesis who developed adenocarcinoma of the endometrium whilst on oestrogen therapy the drug used was diethylstilboestrol. The average duration of therapy was 10·1 years although in one case the drug had been administered for less than five years. The present report indicates that significant hyperplastic changes can be provoked with another oestrogen preparation (ethinyl oestradiol) and that prolonged periods of time are not required for these changes to come about. The effect cannot, therefore, be regarded as confined to diethylstilboestrol alone. Hyperplastic changes can arise even when a low dose of oestrogen is employed although it seems probable that the higher the dose the greater the degree of hyperplasia is likely to be. Duration of administration is also relevant; it seems significant that the eight patients with evident hyperplasia had been treated for 10 years, 4½ years (2 patients), 4 years (2 patients) and 3 years (3 patients) whilst the duration of treatment in those with a simple proliferative pattern was 4 years, 2½ years (2 patients) and 2 years (2 patients).

The characteristics of the endometrium in the seven patients treated with oestrogen and progestogen suggest that this form of treatment is likely to be associated with the development of a more normal type of endometrium; it cannot be concluded from this study that it will be free from risk of the development of endometrial carcinoma but this seems unlikely. Polyp formation in the endometrium was found in one patient in whom the dose of oestrogen whilst not high was higher than is necessary to produce a sufficient oestrogen effect in a patient with gonadal dysgenesis.

All the patients in the study who were receiving unopposed oestrogen therapy have now been placed on an oestrogen/progesterone regime of ethinyl oestradiol 0·02 mg. daily (or its equivalent) for 21 days combined on days 15 to 21 with 5 mg. daily of norethisterone. After some years on this regime or if there is irregular bleeding, curettage is indicated to confirm that there are no changes suggesting the likelihood of the development of endometrial carcinoma.

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# OESTROGEN-INDUCED ENDOMETRIAL CARCINOMA IN A PATIENT WITH GONADAL DYSGENESIS

BY

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## Summary

This case report describes the genesis of endometrial carcinoma after prolonged stilboestrol therapy in a patient with gonadal dysgenesis (Turner's syndrome). The varied histological appearance included the presence of cartilage-like material. A review of the literature reveals the need to treat these patients with combined oestrogen/progestogen preparations rather than with continuous unopposed oestrogens.

## Case Report

An unmarried patient first presented on 26th February, 1970, at the age of 24 years with primary amenorrhoea. She was of short stature and her breasts were poorly developed. Pelvic examination under anaesthesia revealed a rudimentary uterus and no material was obtained at curettage. Cytogenetic studies showed a 45 XO karyotype, confirming the diagnosis of gonadal dysgenesis. Because the patient was concerned about her poor breast development, 0·25 mg. of daily stilboestrol was prescribed.

She was not seen again until four years later when on 9th May, 1974, she was admitted as an emergency with heavy vaginal bleeding. She appeared very ill, with marked pallor, dyspnoea and tachycardia at 150 beats per minute. Pelvic examination now revealed a bulky uterus and confirmed that the bleeding was of uterine origin. The haemoglobin was 5·1 g. per 100 ml. and the serum iron 1·6 $\mu$mol. per l.

A sternal bone marrow examination excluded serious blood dyscrasia and all the haematological findings suggested severe iron deficiency. An electrocardiogram showed sinus tachycardia. A chest X-ray was reported as showing "multifocal lesions throughout the lung fields, probably due to multiple emboli". The blood loss was corrected by the transfusion of four units of concentrated red cells; digoxin, 0·25 mg. daily was administered together with the intramuscular injection of 40 mg. of frusemide. The stilboestrol therapy was discontinued on admission to hospital. Curettage eleven days later produced copious, partly necrotic curettings from a uterine cavity measuring 14 cm.

Histological examination of the curettings showed striking and varied changes, mainly epithelial but including connective tissue features of uncertain significance. There was active epithelial proliferation, ranging from papillary and cystic formations lined by columnar cells (Fig. 1) to more cellular foci of adenomatous hyperplasia which merged in places into solid cellular masses of undifferentiated cells resembling so-called "adenosquamous carcinoma". These latter lacked unequivocal squamous features but apparently squamous foci were present in some other well differentiated glandular areas (Fig. 2). The stroma showed some circumscribed foci of concentrically arranged spindle cells, and elsewhere the stroma seemed generally hyperplastic but lacking definite sarcomatous features. Inflammatory and histiocytic cells were scattered generally, some containing foamy, presumably lipid material. Another finding was the presence of eosinophilic hyaline material, giving negative staining for amyloid. However, most striking were large portions of apparent cartilage (Fig. 3).

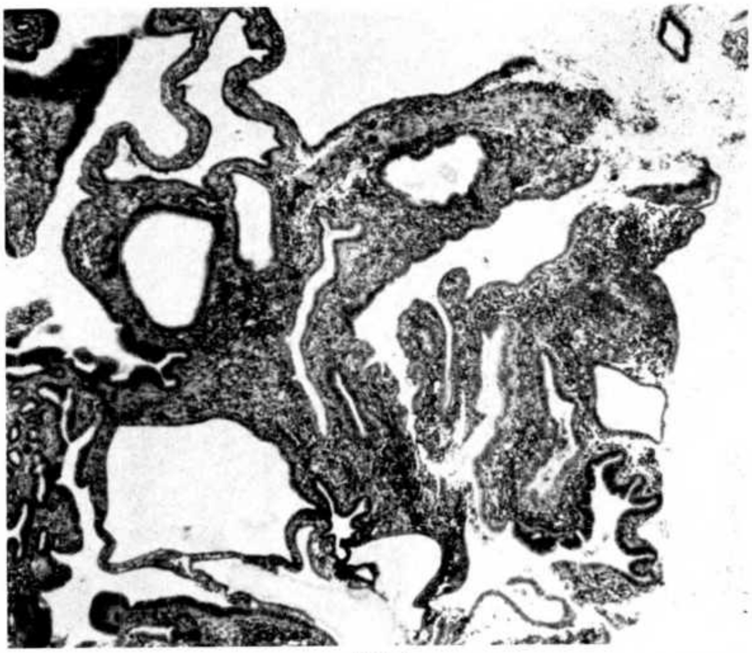

FIG. 1
Benign cystic and epithelial proliferation (Haematoxylin and eosin ×140).

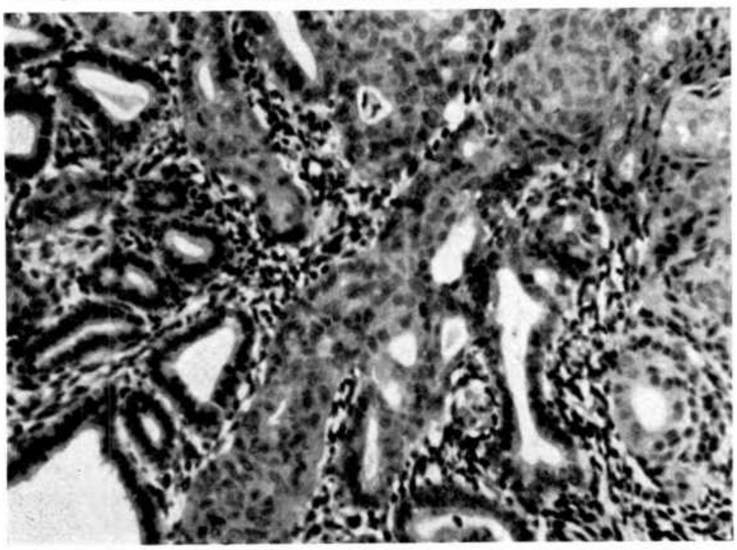

FIG. 2
Adenomatous foci with squamous change (H. and E. ×563).

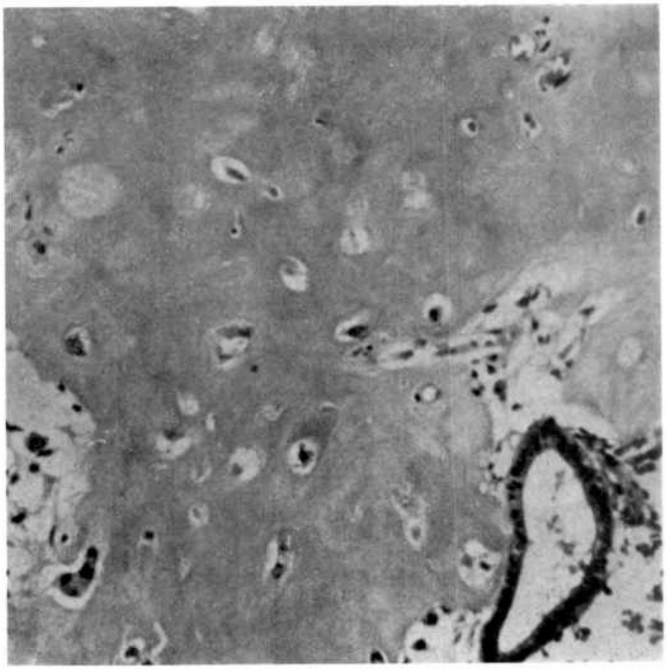

FIG. 3
Cartilage-like change in the stroma (H. and E. × 563).

By 31st May, 1974, there was marked improvement in both the patient's haematological and circulatory state and the X-ray appearances of the chest were now normal. In view of the endometrial histology it was decided to perform hysterectomy. At laparotomy on 10th June, 1974, the uterine body was smaller than had been anticipated from the previous pelvic examination, although larger than in uncomplicated gonadal dysgenesis; the cavity now measured 7·5 cm. There was no external evidence of growth. The Fallopian tubes were thin, and elongated "streak" ovaries were identifiable. Only a scanty partly haemorrhagic residuum of growth was found in the uterine cavity. Histological examination showed changes similar to those seen in the curettings, but there was no penetration of the myometrium. Convalescence was uneventful and the patient was well when last seen on 5th December, 1974.

## DISCUSSION

The histological features of this case are worthy of note. The overall picture was one of extreme variability from simple papillary proliferation to solid carcinomatous portions lacking definite differentiating features although suggestive in places of a squamous origin. However, there were areas of recognizable squamous change in some glandular structures, and these could suffice, taken in conjunction with the other features, to put the lesion in the adenosquamous category (Ng, 1968). This author considers that this is a type of growth which metastasizes early. The presence of cartilaginous foci also deserves attention. It might represent a component of a mixed mesodermal tumour but there was no evidence of the other elements essential for this diagnosis, namely striated muscle fibres, bone and unequivocally sarcomatous tissue. We are not aware that the presence of cartilage in the type of lesion here described has been previously reported and cartilaginous metaplasia of the endometrium is rare (Fox and Langley, 1973). Deep myometrial invasion has been observed in only one case (Cutler *et al.*, 1972), but in the remaining patients there was no evidence of spread beyond the endometrium.

The four-year treatment of our patient was of shorter duration than the average of over five years in other reported cases of oestrogen induced malignant change. One might suspect that the longer the treatment with oestrogens, the more likely is the possibility of myometrial invasion and spread beyond the uterus; and in fact the patient referred to by Cutler *et al.* (1972) had had 20 years' therapy. However, we have not found a report of a fatal case with spread beyond the uterus and bloodborne metastases. Finally, there appears to be universal condemnation of long-term unopposed oestrogen therapy in the management of patients with gonadal dysgenesis and agreement that cyclic oestrogen/progestogen regimes are safer.

## ACKNOWLEDGEMENTS

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# ENDOMETRIAL CARCINOMA AFTER CYCLICAL OESTROGEN-PROGESTOGEN THERAPY FOR TURNER'S SYNDROME

BY

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## Summary

A case of endometrial adenocarcinoma in a patient with Turner's syndrome who had received long term combined oestrogen-progestogen therapy is described.

The development of adenocarcinoma of the endometrium in seven patients with Turner's syndrome after prolonged unopposed treatment with stilboestrol has already been described (see Dewhurst *et al.*, 1975). We now describe what we believe to be the first case of endometrial carcinoma following 13 years of combined cyclical oestrogen-progestogen therapy.

## CASE HISTORY

A patient, then aged 22 years, was investigated in 1959 for primary amenorrhoea and absence of secondary sexual characteristics. Clinical features were typical of Turner's syndrome. She was 133 cm. tall and there was webbing of the neck and cubitus valgus. On gynaecological examination infantile external genitalia, marked uterine hypoplasia and impalpable ovaries were noted. The urinary gonadotrophin assay exceeded 32 mouse units per 24 hours, and cytogenetic studies confirmed a karyotype of 45XO. Therapy was commenced with 0·5 mg. of stilboestrol twice daily continuously for 14 months, and thereafter cyclically in the same dose for the first 21 days of each cycle combined with 5 mg. of ethisterone twice daily on days 16 to 21. This regimen produced satisfactory development of secondary sexual characteristics and regular withdrawal bleeding lasting two to three days. The patient was followed up at six-monthly intervals for four years and then yearly. In July 1973 her haemoglobin was 8·1 g. per 100 ml. and so iron therapy was advised. Four months later she complained that her periods had become heavy and irregular with almost continuous intermenstrual staining, yet her haemoglobin had increased to 11·7 g. per 100 ml. A uterus enlarged to the size of a 20 weeks pregnancy was found

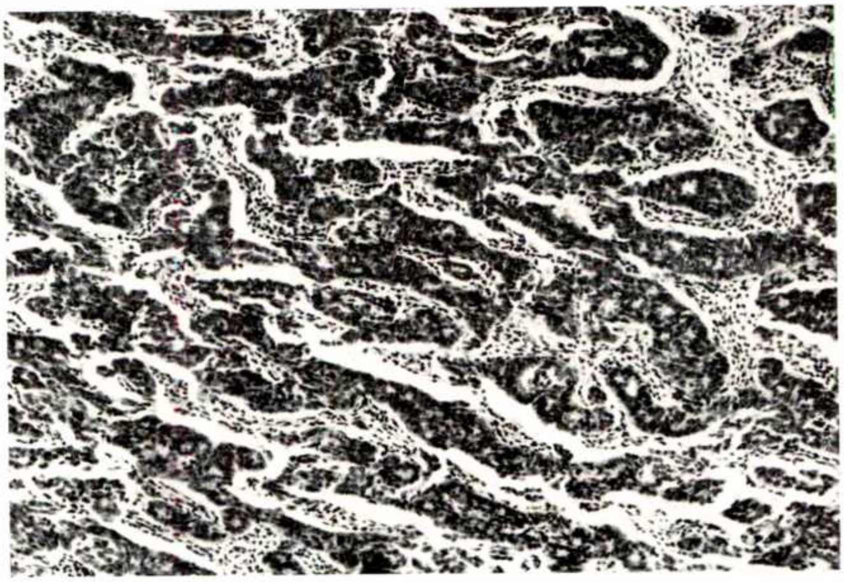

FIG. 1

Adenocarcinomatous proliferation of the endometrium showing a moderately well differentiated tumour. (Haematoxylin and eosin × 110.)

and an endometrial polyp protruding through the cervix was avulsed. Histology showed marked adenomatous hyperplasia with squamous metaplasia. When total hysterectomy was carried out in February 1974 no ovarian tissue was recognized.

The uterus and cervix weighed 170 g. The uterus was bicornuate and had bulky endometrium which histologically showed the presence of a well-differentiated adenocarcinoma with a tendency to squamous metaplasia and osteoid formation in both cornua (see Fig. 1). There was slight infiltration of the myometrium by the tumour but no lymphatic or vascular invasion was noted. The cervix was lined by normal looking squamous epithelium and showed infiltration of the subepithelial zone by chronic inflammatory cells.

## COMMENT

The prolonged unopposed action of oestrogens, whether of exogenous or endogenous origin, has been implicated in endometrial carcinogenesis (Larson, 1954; Jackson and Dockerty, 1957; see Dewhurst *et al.*, 1975). Seven patients with Turner's syndrome have developed this complication following oestrogen therapy of 10·8 years' average duration and at an average age of 30·9 years (see Dewhurst *et al.*, 1975). Further, the histological progression from cystic hyperplasia of the endometrium through atypical hyperplasia to adenocarcinoma has been recorded in women on prolonged oestrogen therapy. Progestogens, however, have been shown to be effective in inducing regression of endometrial adenocarcinoma both *in vivo* and in tissue culture (Eichner and Abeller, 1971; Reifenstein, 1971; Anderson, 1972; Richardson, 1972), and have been recently recommended to mature the "oestrogen-induced" endometrium and prevent hyperplasia (de Koos, 1974). Despite this, our patient on a combined oestrogen-progestogen regimen developed endometrial adenocarcinoma at a comparable age and after a similar duration of therapy as the other cases treated with stilboestrol alone. Her total dose of stilboestrol (3·67 g.) was of the same order as that received by the other cases (mean 4·48 g.) suggesting that this is excessive in this group of patients whether administered alone or combined with progestogen.

Certain abnormal chromosomal constitutions are known to be associated with an increased incidence of malignancy (Wertelecki *et al.*, 1970).

Some cases of Turner's syndrome (45XO) have developed leukaemia (Pawliger *et al.*, 1970; Wertelecki and Shapiro, 1970), and two cases of gonadal tumours in a group of 25 of these patients have been reported (Teter and Boczkowski, 1967), but there is no known association of untreated Turner's syndrome with uterine tumours. A genetic predisposition of patients with X chromosomal abnormalities to this type of cancer cannot be excluded, but oestrogen therapy is likely to have played a causative role in the development of endometrial carcinoma in our patient. Clearly, it is advisable that those receiving such therapy should have periodic gynaecological examinations with curettage even when progestogens are also being used.

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# TOPICAL OESTROGEN THERAPY FOR LABIAL ADHESIONS IN CHILDREN

BY

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## Summary

Labial adhesions separated spontaneously after one month of the topical oestrogen therapy in 22 (88 per cent) out of 25 children. This treatment was safe, painless, without significant complications and of lasting value.

ADHESIONS of the labia minora are not uncommon in children. Local inflammation and oestrogen deficiency are thought to be important aetiological factors. This condition is usually harmless and resolves spontaneously at puberty (Anderson, 1956). However, severe cases should be treated to correct urinary problems or to relieve parental anxiety. Several authors have recently advised topical oestrogen therapy (Huffman, 1968; Altchek, 1972; Dewhurst, 1972; Capraro and Greenberg, 1972). However, the results of this method are uncertain and the purpose of this paper is to report on the use of topical oestrogens in 25 children with severe adhesions of the labia minora who were seen during a 4-year period at Chulalongkorn Hospital, Bangkok.

## PATIENTS AND METHODS

Twenty-five children between the ages of 2 months and 2 years were treated. The adhesions were in all cases severe, involving the entire length of the labia minora. A tiny opening through which urine passed was usually present just underneath the clitoris. Ten patients were symptom-free and fifteen had either frequency of micturition or dysuria or both. Four children were noticed to have leakage of residual urine from the vagina; two had urinary tract infections and one had a co-existing vulvovaginitis. One patient had one year previously had surgical treatment for labial adhesions. All patients had normal external genitalia at birth.

The parents were told to apply to the adhesions 0·01 per cent dienoestrol cream every night while the children were asleep. For control purposes, five patients were treated with a bland ointment instead of the oestrogen cream. During treatment the children were seen weekly at the outpatient clinic, and after treatment they were examined at regular intervals for at least one year.

## RESULTS

In 22 out of 25 children adhesions of the labia minora separated after topical oestrogen therapy. In those treated with success the duration of treatment was one week in eight patients, two weeks in ten patients, three weeks in three patients and four weeks in one patient. Only partial separation of the labia occurred in one patient treated for six weeks and two patients were treated for eight weeks without any success, after which the adhesions were separated under anaesthesia.

Slight vulval pigmentation occurred in practically every patient, but it gradually disappeared after the oestrogen medication was stopped. One child had erythema of the vulva due to excessive use of oestrogen cream. No systemic side effects were seen. During follow-up, one patient had a recurrence of adhesions two months after successful treatment. Both patients treated surgically had a recurrence within two years. All recurrent adhesions responded readily to further local oestrogen therapy.

The children who were given bland ointment for one month showed no change. However, all were subsequently treated successfully by topical oestrogens.

## DISCUSSION

Labial adhesions which cause urinary complications or parental anxiety should be treated. Otherwise they should be left to separate by themselves at puberty when there is a rise in endogenous oestrogen production.

Labial adhesions used to be treated by blunt or sharp dissection (Bowles and Childs, 1943; Nowlin *et al.*, 1949); this method is painful, may be complicated by bleeding or dysuria and recurrence rates were reported as 20 per cent by Nowlin *et al.* (1949) and 100 per cent by Capraro and Greenberg (1972). Both the children who we treated surgically had a recurrence of adhesions within two years.

Using topical medication similar to ours, Capraro and Greenberg (1972) reported a success rate of 90 per cent. However, the adhesions in some of their patients were not severe. Huffman (1968), using 0·1 per cent oestrogen cream for 10 to 14 days claimed to have no failures. Labial adhesions have also been treated by oral oestrogens (Teton and Treadwell, 1956). Bland ointment produced no change, indicating that mechanical pressure is not enough to separate adhesions.

Oestrogen cream may cause vulval pigmentation or erythema. The duration of treatment should not be more than eight weeks because it is unlikely to succeed after that and is more likely to produce systemic side effects.

Recurrence of adhesions seldom occurs with hormone therapy but bland baby ointment or cortisone cream applied locally for several weeks after oestrogen therapy may help to prevent this complication (Capraro and Greenburg, 1972).

## ACKNOWLEDGEMENTS

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# PHAEOCHROMOCYTOMA IN PREGNANCY

## CASE REPORT

BY

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### Summary

A patient was found to have a phaeochromocytoma in the 36th week of pregnancy. She was successfully managed by medical treatment, vaginal delivery and resection of the adrenal tumour seven weeks after delivery.

### Case Report

The patient was aged 25 years and was in her second pregnancy, the first having miscarried at 16 weeks gestation two years earlier. When she booked at 13 weeks her blood pressure was 135/85 mm. Hg. and there was no proteinuria. The blood pressure remained below 135/85 mm. Hg., and the pregnancy was normal until 36 weeks when a blood pressure of 140/100 mm. Hg. was noted. There was no associated proteinuria and she was admitted on 9th September 1974 for further investigations.

The patient was tense, agitated, and excitable but abdominal examination revealed a normal 36 week gestation. Examination of the retina showed arteriolar spasm without haemorrhages. There was no glycosuria or proteinuria. A random blood sugar was 67 mg./dl., the blood urea was 20 mg./dl., the serum creatinine 1·1 mg./dl., the urine creatinine 57·9 mg./dl., and the creatinine clearance 107 ml./minute.

Three days after admission marked fluctuations were noted in the blood pressure, with minimum and maximum levels of 140/90 mm. Hg. and 210/110 mm. Hg. Complaints of frontal headaches, photophobia, epigastric pain and hot flushing were noted during the hypertensive episodes which occurred three to four times in 24 hours. The temperature was normal, pulse rate 88 per minute and respiratory rate 30 to 40 per minute. Palpation of the fetus, bed bathing and other movements of the patient were noted to precipitate attacks of hypertension.

A semiquantitative test for adrenaline and noradrenaline in urine (Hingerty, 1957) was strongly positive and the urinary excretion of 4-hydroxy-3 methoxy mandelic acid was found to be 40·8 mg./24 hours (normal less than 7 mg./24 hours).

On 20th September 1974, and in the 37th week of pregnancy, the patient developed gross albuminuria, and a diagnosis of pre-eclampsia complicating phaeochromocytoma in pregnancy was made. Three serial urinary oestriol estimations had been within normal limits and it was decided to deliver the baby as soon as the peripheral effects of the tumour secretions could be blocked.

An intravenous infusion of phenoxybenzamine hydrochloride, in 5 per cent dextrose solution, in a dose related to the patient's weight was administered for two hours, with 5-minute pulse and blood pressure recordings (Ross *et al.*, 1967). Forty minutes after commencement of the first infusion a pulse rate of 140 per minute was noted, and a loading dose of 400 mg. of practolol was administered orally followed by eight hourly doses of 200 mg. The phenoxybenzamine infusion was repeated every 24 hours for three days. On this regime the blood pressure became normal, the proteinuria disappeared and the patient's symptoms abated.

On 23rd September 1974, labour was induced by low and high amniotomy supplemented by an oxytocin infusion when contractions had not commenced 12 hours later. The first stage of labour lasted nine hours and a healthy male infant, weighing 3·82 kg., was delivered. An episiotomy was performed to aid the second stage and 0·5 mg. of intravenous ergometrine was administered with the birth of the baby. The patient was given either pethidine or omnopon every four hours from the time of amniotomy until delivery. The highest blood pressure recorded was 140/94 mm. Hg. and the pulse rate never rose above 90 per minute. The blood pressure immediately following delivery was 130/80 mm. Hg., and the pulse rate was 80 per minute. Following delivery, the patient continued on 200 mg. of practolol and 20 mg. of phenoxybenzamine three times a day orally. The blood pressure and pulse rate remained within normal limits. Some episodes of postural hypotension in the first week after delivery necessitated gradual ambulation. An intravenous pyelogram performed six days *post partum* showed the right kidney to be displaced downwards and rotated laterally with a mass immediately above it. The patient was discharged home on the above medication 12 days after delivery. Right trans-femoral arteriography carried out at five weeks after delivery confirmed the presence of a relatively vascular oval mass at the upper pole of the right kidney, mainly supplied from the renal artery and a suprarenal branch arising from the aorta.

Tumour resection was carried out successfully seven weeks *post partum* on 14th November 1974 and, apart from a small reactionary haemorrhage from the loin incision, the patient had an uneventful postoperative recovery. Urinary catecholamine excretion returned to normal and the blood pressure was 130/80 mm. Hg. two days after stopping all drug therapy.

The tumour was encapsulated, greyish-brown in colour, cystic in places and measuring 12·0×9·0×8·0 cm. The cut surface had a glazed brownish-grey and in places haemorrhagic appearance. The circumference of the tumour showed a yellowish streak of tissue. The histology confirmed the tumour to be a phaeochromocytoma containing large areas of haemorrhagic necrosis.

## DISCUSSION

Existing obstetric literature gives no positive guide to the management of the diagnosed case. Most authors stress the dangers of attempting to localize the tumour during pregnancy by radiological techniques which can produce fatal crises (Bennett and Mather, 1959; Koonce *et al.*, 1962; Blair, 1963; Fox *et al.*, 1969; Smith, 1973) and expose the fetus to irradiation.

Treatment with drugs to block the peripheral and central vascular effects of the tumour secretions is an essential preliminary part of management at all stages in pregnancy. Phenoxybenzamine reduces the blood pressure by occupying the peripheral vascular alpha receptor sites for catecholamines, while practolol reduces the pulse rate by similar competitive blockade at beta receptor sites in the heart.

The optimum form of subsequent obstetric and surgical management is not made clear by study of previous reviews. If the diagnosis is made in the first two trimesters, Smith (1973) suggests excision of the tumour and accepts the risk of spontaneous abortion (Helps *et al.*, 1955). It may, however, be possible to defer definitive surgical treatment by drug therapy until the fetus is viable.

When the diagnosis is made in the third trimester several authors recommend delivery by combined elective Caesarean section and resection of the adrenal tumour (Fox *et al.*, 1969; Donald, 1972). A successful outcome with such management has been reported by Barzel *et al.* (1964), Smith (1973) and Dean (1958). Others have favoured removal of the tumour some weeks after Caesarean section (Maloney, 1955).

The present case demonstrates the possibility of achieving a normal delivery in pregnancy complicated by phaeochromocytoma if the peripheral effects of circulating catecholamines can be blocked by drugs. The tumour can then be removed in the puerperium by a procedure which is safer because the vascularity associated with pregnancy is absent, access to the tumour is easier, there is no risk of disturbance of the tumour by preliminary Caesarean section, and accurate study of the tumour's location and vascularity by pyelography and arteriography has been possible.

## ACKNOWLEDGEMENTS

We are indebted to Mr. J. Girling, Dr. A. D. C. Norris and Miss F. A. Musson for help in the management of our patient.

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*British Journal of Obstetrics and Gynaecology*
May 1975. Vol. **82**. pp. 429–430.

# BOOK REVIEWS

**Antenatal Education. Guidelines for Teachers.** By M. WILLIAMS and D. BOOTH. Pp. x+178 with 10 figs. Churchill Livingstone, Edinburgh, 1974. Price: £1.75.

THIS very interesting paperback volume sets out guidelines, information and detailed instructions to those people who wish to teach women about their pregnancy and its management. It contains simple yet invaluable principles of teaching techniques, not to be found in ordinary medical textbooks, as well as contents of the actual education programme. Its message is clear, precise and well presented. As the standards of care rise in obstetrics, mothers are demanding more of the maternity services themselves. This is expressed in an increased demand for "natural childbirth", "relaxation classes", "psychoprophylaxis", "preparation for childbirth". It scarcely matters what it is called. Mothers want to know what is going on, they have a right to know, they are actively involved and it is certainly time that the professions who have the responsibility for the care of pregnant women accepted the fact that their patients have been inadequately informed for far too long. Professor Rhodes, in the foreword, says "In the present climate of opinion it is no longer necessary to defend antenatal education". Whilst this remark may be correct in certain spheres, there are still many places including hospitals and many people including doctors to whom it is necessary to defend antenatal education. Such education presents an enormous problem. It is time-consuming and requires small group teaching. This book is a genuine, thorough and indeed excellent introduction into the practice and theory of such teaching. It should be read and understood by all involved in antenatal teaching. Since most obstetricians are associated with antenatal teachers and since all of them ought to be undertaking at least some elements of antenatal teaching for their own patients, then they would all benefit from reading this book. All those who are engaged in the teaching of either undergraduates or postgraduate students or in the training of midwives would find some of the principles of teaching contained in this volume to be of immense value and its enlightened approach and humane philosophy would make them more aware of the requirements of their patients and would therefore increase not only the standard of their teaching programme, but also the quality of their clinical management.

*G. Bourne.*

**Illustrated Human Embryology. Volume 3: Nervous System and Endocrine Glands.** By H. TUCHMANN-DUPLESSIS, M. AUROUX and P. HAEGEL. Pp. xii+143. Chapman & Hall, London, 1974. Price: £4.50.

THIS volume completes the set of three that make up this text of Illustrated Human Embryology. Embryogenesis and general organogenesis were dealt with in Volumes I and II. This volume considers the development of the nervous system, the sensory organs and the endocrine glands. The text is clear, the illustrations are bold and sensible and consist of clearly labelled line diagrams, photomicrographs and three-dimensional reconstructions. The detail is accurate and there is little to criticise. Each chapter includes an analysis of the timing of the various stages of development and summarizes the principal malformations found in man. By combining descriptions of normal embryology with notes on clinical application the undergraduate and the clinician will find it helpful in the understanding of congenital abnormalities.

*J. M. Davison.*

**Perinatal Pharmacology. Problems and Priorities.** By JOSEPH DANCIS and JOSEPH C. HWANG. Pp. xii+228, with 57 figs. and 42 tables. Associated Scientific Publishers, Amsterdam, 1974. Price: Dfl. 60.00 US $23.10.

THE title of this volume may lead to misunderstanding. Only 8 of the 22 contributors have attachments to departments of paediatrics and only one to an obstetric department. Though much of the work presented bears indirectly on perinatal pharmacology, there is little of direct interest to the paediatrician or obstetrician concerned with clinical perinatal care. For that matter, there is very little classical pharmacology, most of the material dealing with biochemical and metabolic studies of tissues from pregnant animals. In the final section, Dr. Mirkin gives a three page list of the research goals of developmental pharmacology. Some of the present contributions are basic biochemical studies which may eventually prove to be relevant to these aims. The report of Hahn and Kirby on enzyme induction in human fetal liver isolated in culture is an example. Other topics, such as the observations of Smith, Hui and Crafford on the effects of drugs on limb regeneration in the salamander, deal with aspects which at present seem quite isolated. It is clear that there is much need for further co-ordination of studies in this field.

*D. F. Hawkins.*

**Practical Human Cytogenetics.** By ANGELA I. TAYLOR. Pp. x+179, with 81 figs. Baillière Tindall, London, 1974. Price: £3.00.

THIS small book is a useful and practical introduction to accurate clinical diagnosis and reliable laboratory procedure in the field of applied human cytogenetics. Dr. Taylor deals with the problem of clinical recognition of the chromosomally abnormal infant by providing clear, well illustrated and simple accounts of the phenotypes of the major autosomal trisomies. She also tabulates the diverse clinical findings in patients with specific structural abnormalities of the autosomes. The chapter on dermatoglyphics and their practical value in ascertaining the clinical diagnosis is excellent. The second problem she tackles is the establishment of the diagnosis in the laboratory. She describes most accurately the problems that may be encountered when analyzing karyotypes and identifying sex chromatin in buccal smears; the advice she gives for solving them is sound. Regrettably, she has not included in this book the techniques of cell culture and harvesting; she does not discuss, for example, the causes of failure of the chromosomes to spread or the reasons for poor chromosome detail. Such practical and experienced advice could be of value to the small laboratory or the isolated worker who cannot immediately receive expert analysis of their problems. Other chapters include a useful account of variation in the normal human karyotype and short descriptions of the major sex chromosome anomalies. There are practical notes on antenatal diagnosis of chromosomal disorders and on sex chromatin surveys of newborn infants. The observations on the clinical significance of mosaicism and translocation in Down's syndrome are particularly well expressed. The book is well presented and clearly indexed with good illustrations and diagrams. It is an important handbook for technicians and may be informative to others whose work brings them into contact with the problems of human cytogenetic aberrations.

*D. Coleman.*

**Carbohydrate Metabolism in Pregnancy and the Newborn.** By H. W. SUTHERLAND and J. M. STOWERS. Pp. 252. Churchill Livingstone, Edinburgh, 1975. Price: £8.00.

BOOKS which comprise papers presented at a symposium are often of limited value; there can be considerable overlapping of subjects and not everyone who presents a paper at such a meeting necessarily has anything significant to say. In the preface to this book the editors clearly state that the contents are the result of a Colloquium and thus form "an assembly for discussion" (Oxford English Dictionary). However those so assembled were largely members of the Diabetic Pregnancy Study Group which holds an annual "workshop" into the problems of carbohydrate metabolism both in the pregnant woman and the newborn. Each is actively involved in research into carbohydrate metabolism and its disorders, as well as the day-to-day clinical management of diabetic patients. There are six major sections starting with the physiological aspects of carbohydrate metabolism and ranging through the whole spectrum of fetal pancreatic behaviour, the assessment of the feto-placental unit, the management of the newborn and many aspects of the detection and management of the diabetic mother. The contributors come mainly from Western Europe and this reflects the increasing trend towards active cooperation of doctors throughout Europe when investigating complex problems. No book contains "imperishable truth" and some readers will find their own opinions concerning the detection and management of the diabetic mother at variance with those expressed by the authors. However, the book is up-to-date and brings together in one volume virtually all aspects of the management of diabetes during pregnancy. If there is a criticism it is that the print is small and there is inadequate spacing between the tables and text. Nevertheless the book can be recommended to colleagues in all disciplines connected with the management of the pregnant diabetic patient and her infant.

*T. Lind.*

*British Journal of Obstetrics and Gynaecology*
May 1975. Vol. **82**. pp. 431–432.

# CORRESPONDENCE

## Treatment of Stress Incontinence by a Fibrin Bioplast.

HORN, B., KOVER, J. and MARTON, I. (1975): *British Journal of Obstetrics and Gynaecology*, **82**, 61.

*From:* G. T. HAMMOND

Sir,

Your readers may be interested to hear that the operation described by Horn *et al.* (1975) has been performed in Winchester since March 1973. A total of 127 patients has been treated to date and I now briefly report the results obtained in the first 62.

Four of them (6.5 per cent) failed to attend for follow-up and all four were completely cured at the time of discharge from hospital. The results obtained in the other 58 patients are shown in the Table where they are grouped according to duration of follow-up, result of operation and initial severity of stress incontinence.

It can be seen that of 58 patients seen post-operatively 52 (89·7 per cent) were cured. These figures are similar to those of Horn *et al.* (1975) and suggest the treatment of stress incontinence by a fibrin bioplast can give results which are at least as good as those obtained with more complicated procedures.

The simplicity of the technique is well shown in the illustrations in the article by Horn *et al.* (1975) and they were drawn by Mr. Peter Jack of the University of Southampton from an operation seen at Winchester.

Yours etc.,
G. T. HAMMOND,
*Royal Hampshire County Hospital, Winchester*

| Duration of follow-up | Result | Initial severity of stress incontinence | | | Total cured |
|---|---|---|---|---|---|
| | | Severe | Moderate | Slight | |
| 12 to 18 months (27 patients) | Cured | 10 | 12 | 2 | 24 |
| | Partly cured | 3 | | | |
| 7 to 11 months (21 patients) | Cured | 14 | 6 | 1 | 20 |
| | No improvement | | | 1 | |
| Up to 6 months (10 patients) | Cured | 3 | 5 | | 8 |
| | Partly cured | 1 | | 1 | |
| | | | | | 52 |

## Rapid Enlargement of a Uterine Fibroid after Clomiphene Therapy.

FRANKEL, T., and BENJAMIN, F. (1973): *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **80**, 764.

*From:* J. E. FELMINGHAM and R. CORCORAN

Sir,

Frankel and Benjamin (1973) reported a case in which, after clomiphene therapy for anovulation, one of several fibroids previously seen at culdoscopy grew rapidly in size. They advised that the drug be administered with caution in patients with fibroids.

We have recently encountered a similar case which indicates that even if fibroids are not known to be present before treatment, the differential diagnosis of an abdominopelvic mass after clomiphene must include rapid enlargement of a fibroid as well as ovarian enlargement due to hyperstimulation and pregnancy.

Our patient (aged 30 with four years of primary infertility due to anovulation with irregular periods) received four 5-day courses of clomiphene over a period of five months, the daily dose of clomiphene being 50mg. in the first course and 100mg. thereafter. Six weeks after the completion of this treatment a fibroid on the posterior uterine wall, which had not been palpable bimanually, grew to measure 10×10×11 cm. Neither culdoscopy nor laparoscopy had been performed before clomiphene was given.

Yours, etc.,
J. E. FELMINGHAM and R. CORCORAN,
*Wallasey Hospital for Women,*
*Wirral, Merseyside*

**Utero-abdominal Sinus After Classical Caesarean Section.** JAIN, S. P. (1974): *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **81**, 333.

*From:* B. P. PUJARI

Dear Sir,

The author of the above case report gives the impression that the literature contains no reference to the occurrence of a utero-abdominal sinus after classical Caesarean section. In fact, various authors have reviewed utero-abdominal fistulae and have stated that the commonest predisposing factor was classical Caesarean section (Poddar, 1951; Ballin, 1910; Kirkland, 1959; Murata and Maruyama, 1960; Yoshimura *et al.*, 1960). Recently we saw a utero-abdominal fistula following *lower* segment Caesarean section and we have been able to trace only one similar case report (Kirkland, 1959).

Our patient presented at the age of 22 with an abdominal sinus from which blood-stained discharge occurred at the time of menstruation. She had had two previous pregnancies. The first ended in 1969 in a lower segment Caesarean section for placenta praevia. Following this she had wound sepsis and developed an incisional hernia. A swelling attached to the uterus was palpated during the second pregnancy which ended in a normal delivery in August 1972. One month later the swelling burst leaving, in the lower part of the incisional hernia, a sinus which at first discharged serous fluid intermittently. At operation this sinus led into a fistulous track which ended in the lower segment scar. The fistulous track was excised and the uterus was closed with No. 1 chromic catgut. The incisional hernia was repaired and the patient made a good recovery, no fistula or incisional hernia being present one year later.

Yours etc.,
B. D. PUJARI,
*Shree Hospital,*
*Miraj, India*

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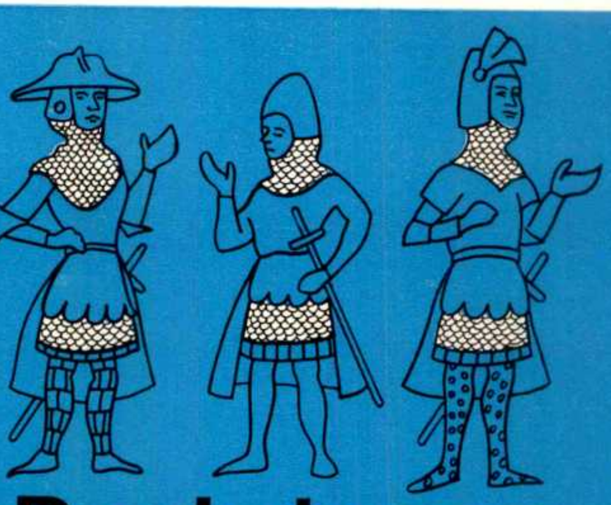

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"*Progress:* The patient continued to improve", NOT "*Progress:* Went downhill".

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The author's name and his appointment at the time of preparation of the text should appear at the head of the paper.

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All tables and illustrations should be separated from the text, but with their positions indicated. Tables are numbered with Roman numerals and figures with Arabic numerals. Authors may be required at the Editor's discretion, to contribute to the cost of reproducing tables and illustrations if these are very numerous. It is regretted that the high cost of coloured plates cannot be met by the Journal. Estimates of cost can be obtained.

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Recognizable photographs of patients must be accompanied by written permission to publish from the patient or her relatives.

## SI UNITS

Wherever possible, authors should express measurements in SI units and whenever such a measurement appears in the text of an article it should be followed in parentheses by the equivalent in traditional units. Tables and figures should be presented in SI units, conversion factors to traditional units being given as footnotes. Those seeking guidance should refer to their local laboratories or to an article by Professor D. N. Baron at page 509 of the *British Medical Journal* of 30th November, 1974.

## ABBREVIATIONS

These should be avoided as far as possible. "Per cent" should be used instead of the symbol "%" and such forms as T.B., R.B.C., B.P., P.E.T., A.P.H. eschewed. Dates should be given as 10th May, 1974 and *not* 10.V.74. The official rather than the proprietary names of drugs must be used.

The Journal convention is C for Celsius, m for metre(s), g for gramme(s), A for ampere(s), k for kelvin(s), cd for candela(s), m for mole(s), l for litre(s) and "/" for the divider "per". Submiltiples of units are formed by prefixes and authors should use d for deci or $10^{-1}$, c for centi or $10^{-2}$, m for milli or $10^{-3}$, $\mu$ for micro or $10^{-6}$ n for nano or $10^{-9}$, p for pico or $10^{-12}$, f for femto or $10^{-15}$ and a for atto or $10^{-18}$; for multiples the prefixes should be T for tera or $10^{12}$, G for giga or $10^{9}$ M for mega or $10^{6}$ k for kilo or $10^{3}$ h for hecto, or $10^{2}$ and da for deca or $10^{1}$. Thus it would be kg. for kilogramme(s), ml for millilitre(s), $\mu$g for microgramme(s), etc. Symbols should be typed without a full stop. In the writing of large numbers, groups of three figures should be separated by a space and not a comma (to avoid confusion with decimal points).

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Brown, H. E., and Jones, B. K. (1961): *Journal of Obstetrics and Gynaecology of the British Commonwealth*, **2**, 128.

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1. Obstet. Gynec. (1966) 28:224-232.
2. 'Pain' (1972) Churchill Livingstone, London. pp. 395-397.
3. Brit. J. Anaesth. (1974) 46:365-372.

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1. Brit. Med. J., (1973), **4**, 64

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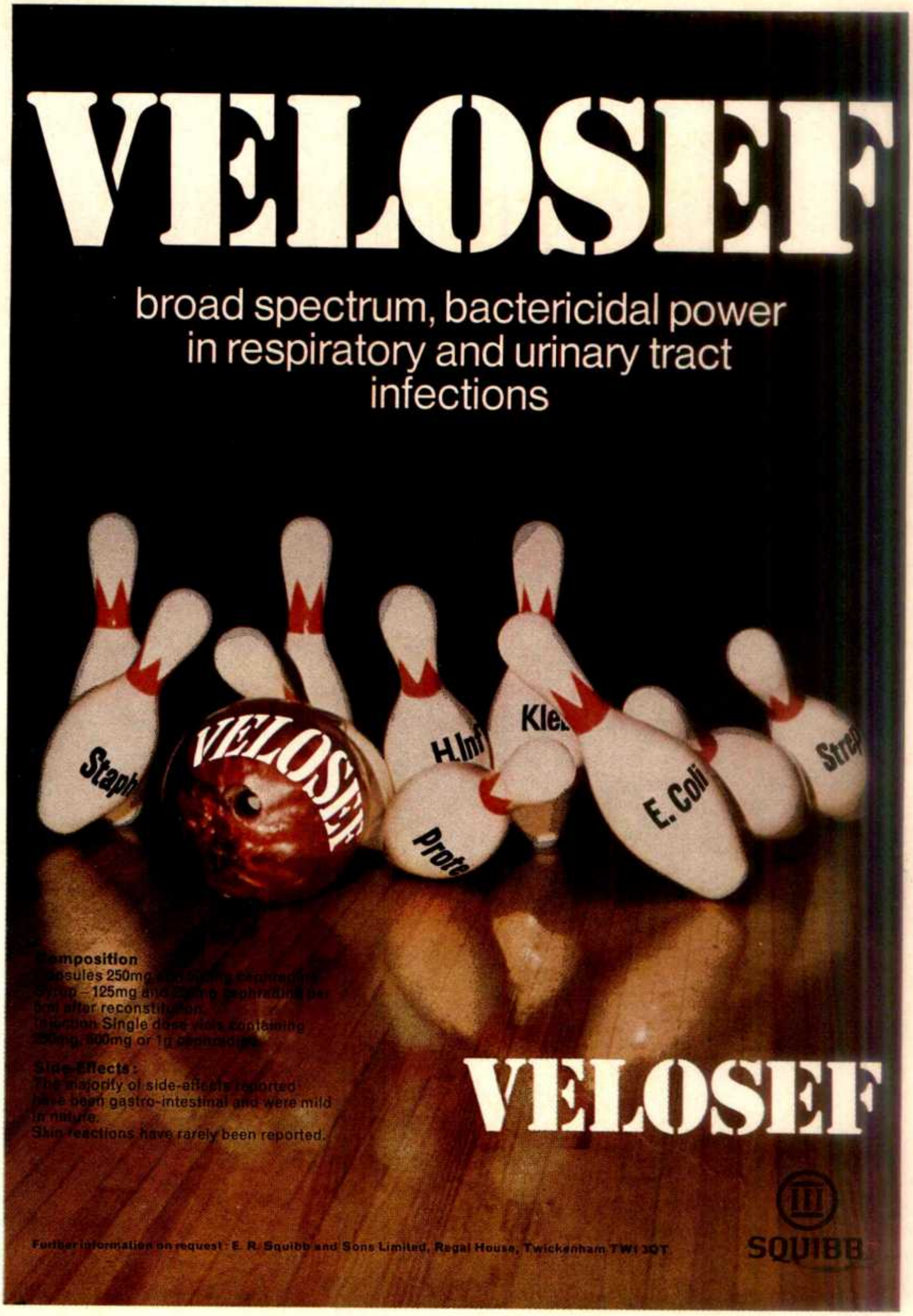

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J.Obstet.Gynaec. Brit. Cwlth. 1972. **79.** 517.

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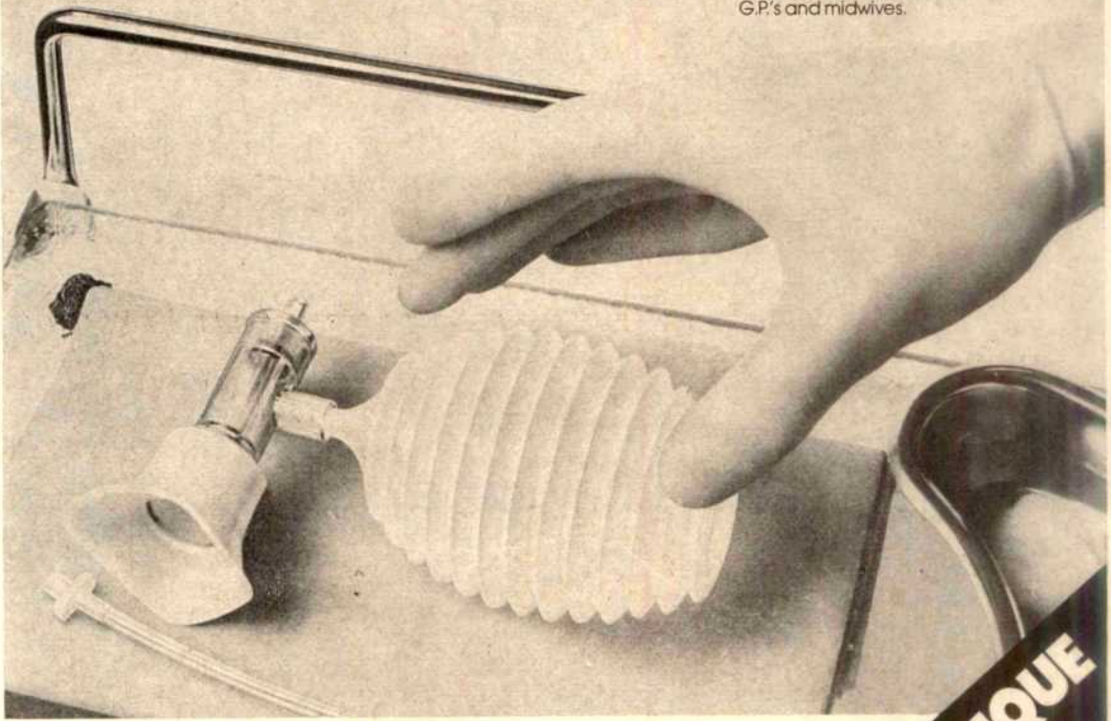

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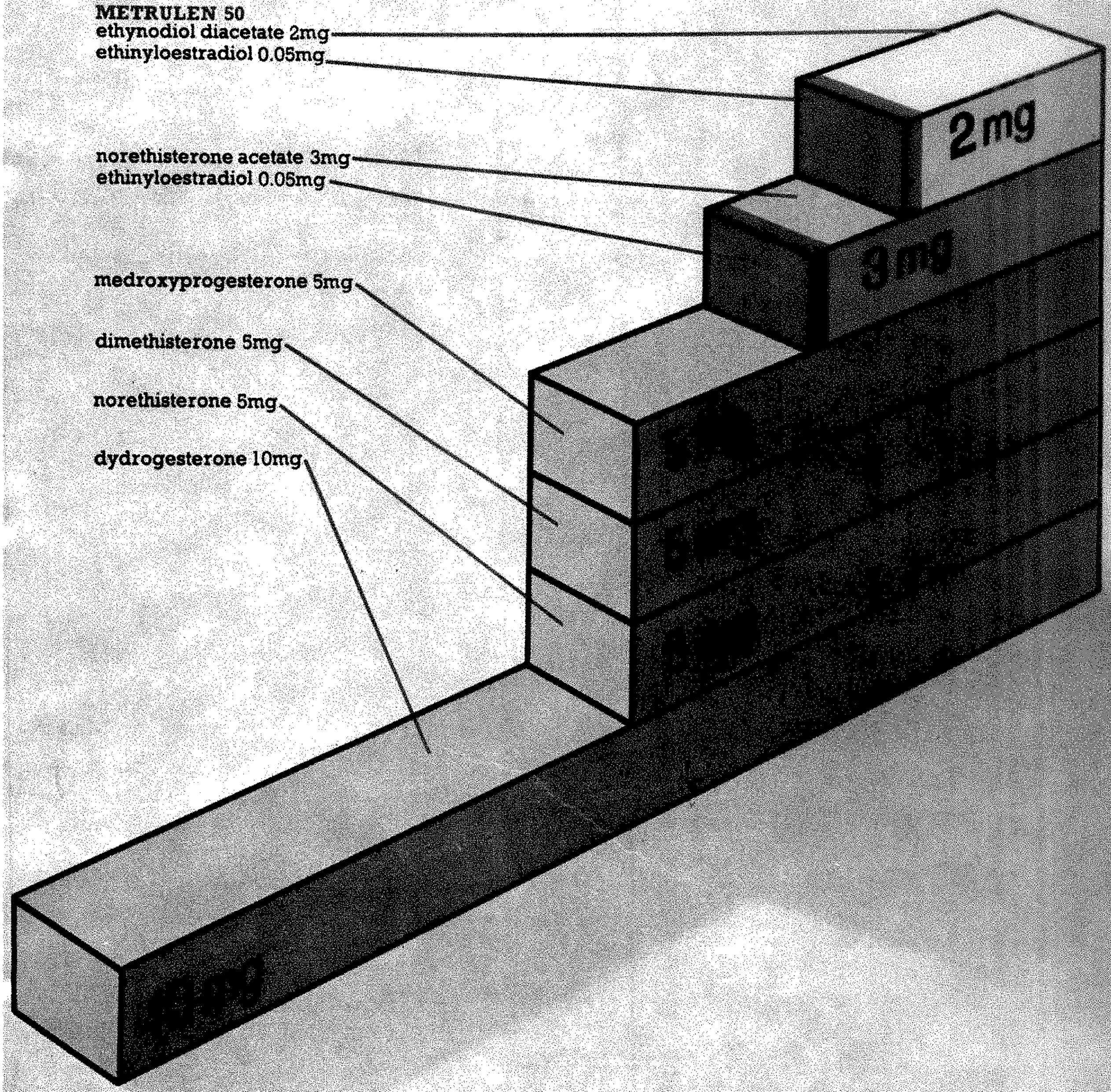

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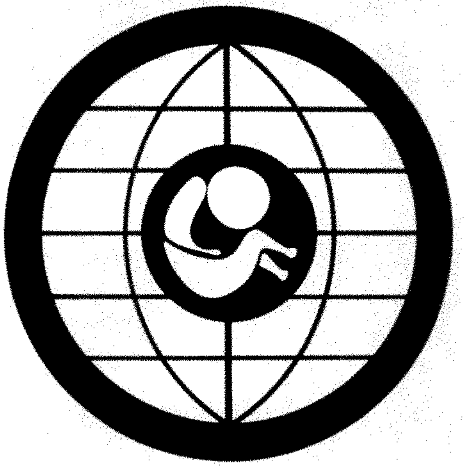

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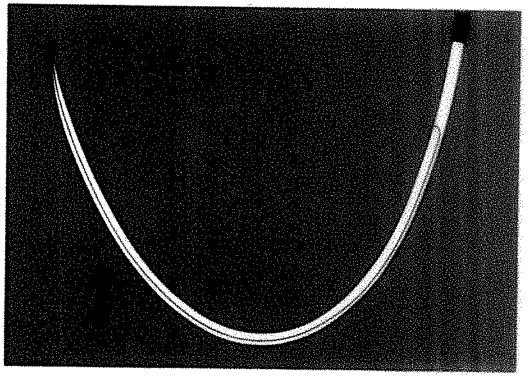

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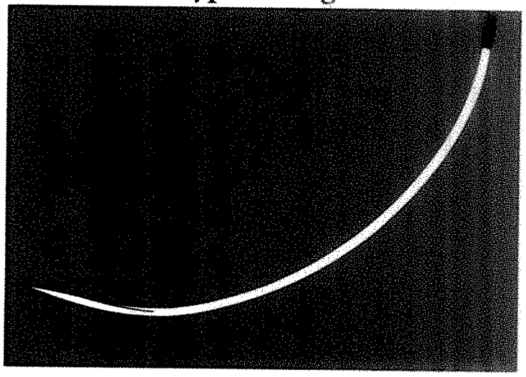

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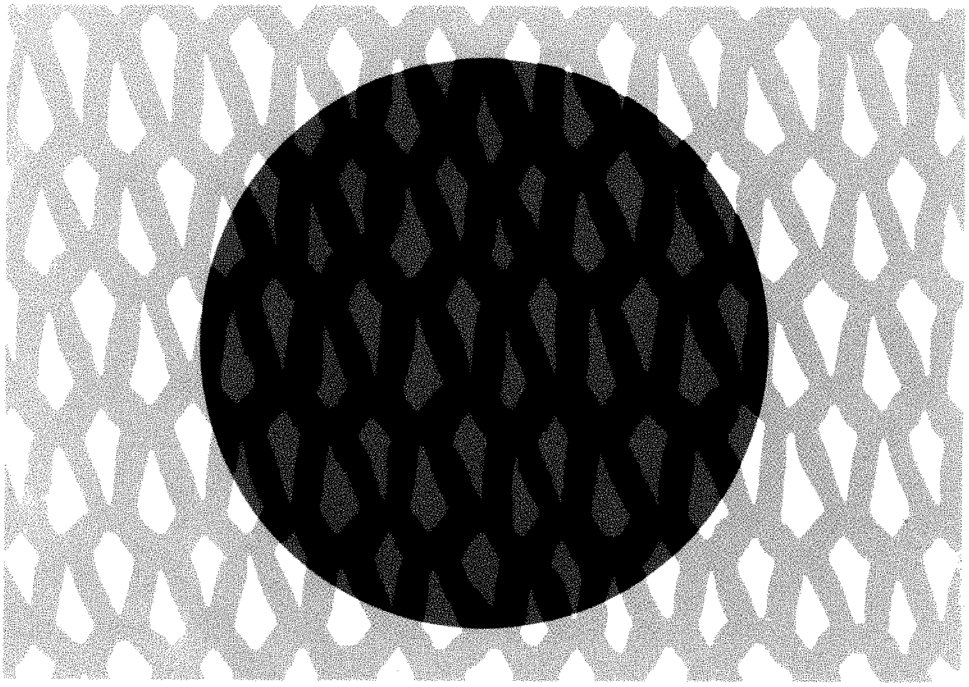

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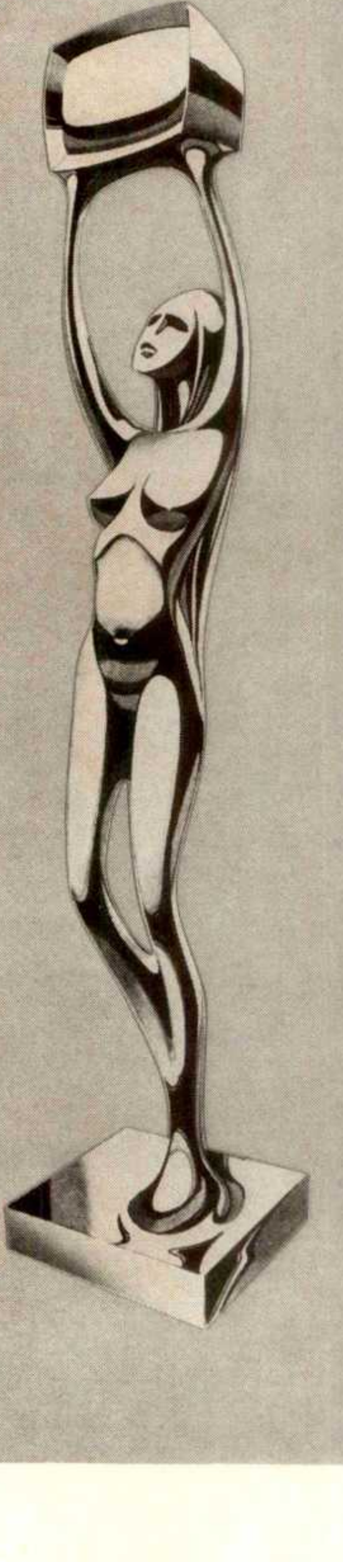

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1 **World Med., 8,** (19), 73, 1973
2 **Gen. Practit.,** p. 19, 13 July, 1973
3 **Practitioner, 212,** 115, 1974
4 **Br.Med.J., iv,** 146, 1971
5 **Ibid., i,** 207, 1971

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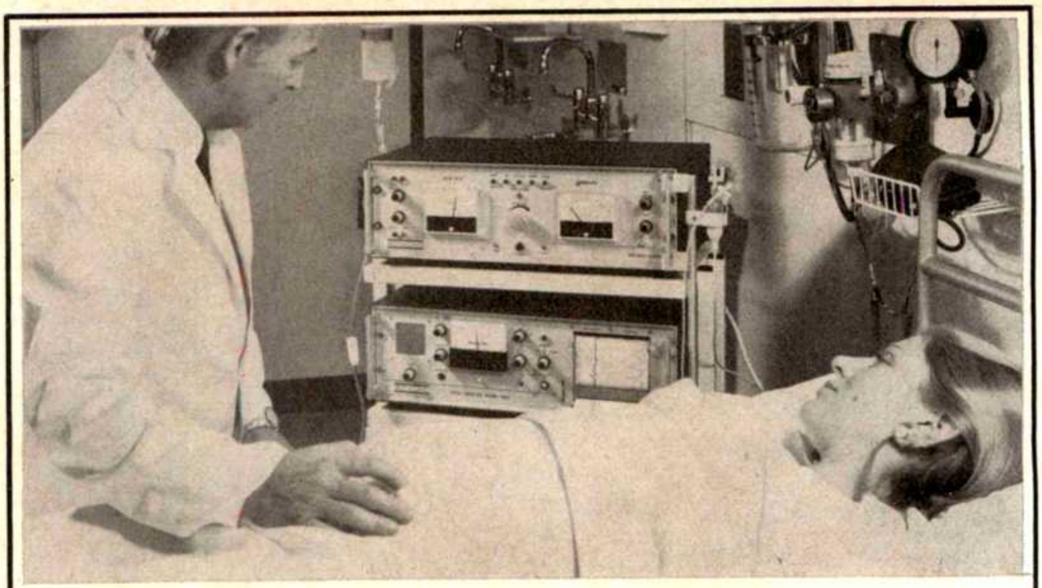

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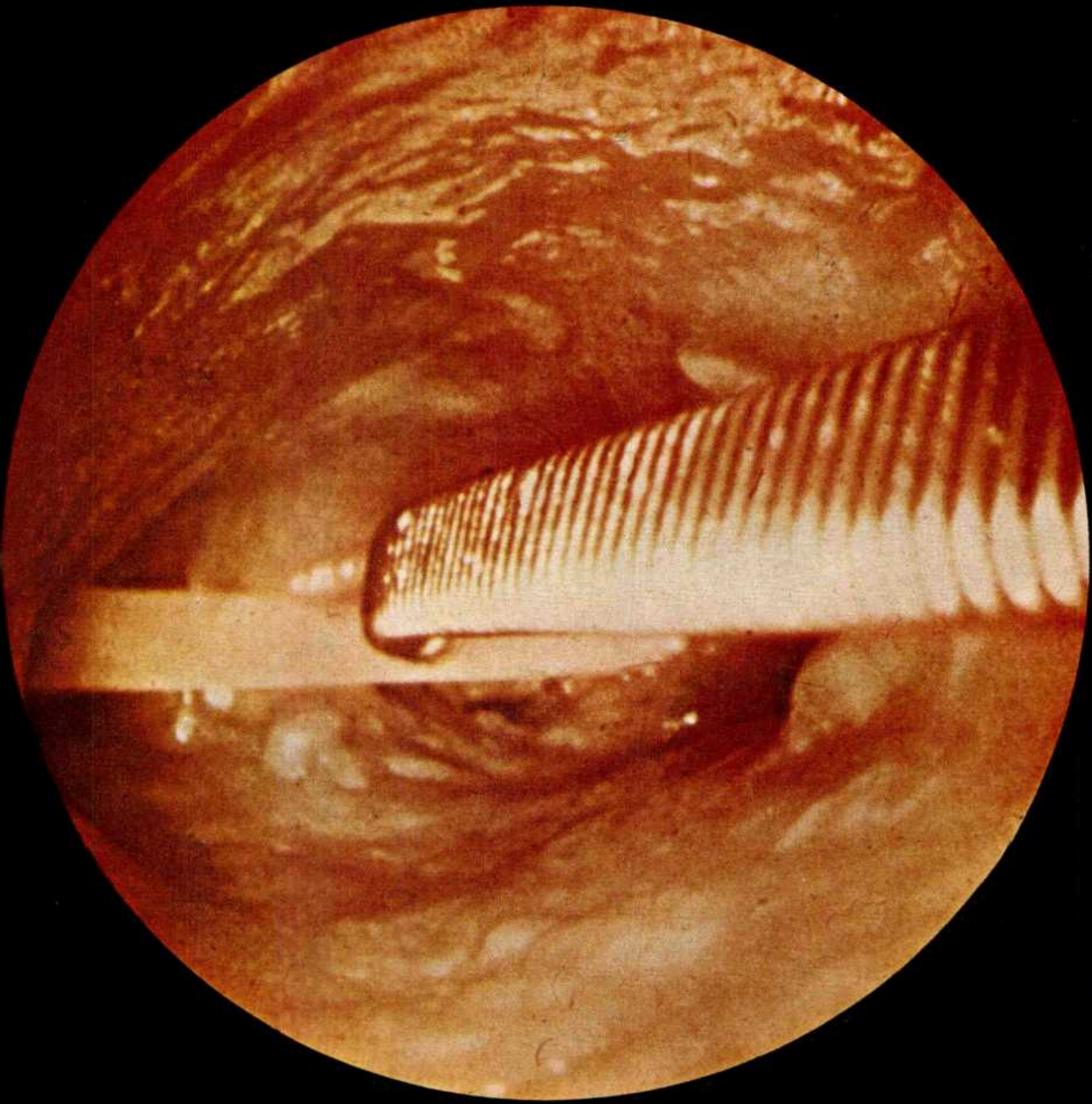

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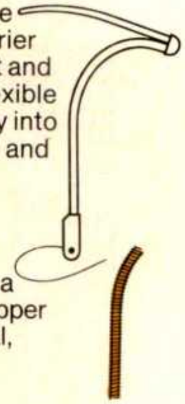

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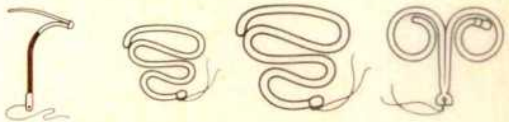

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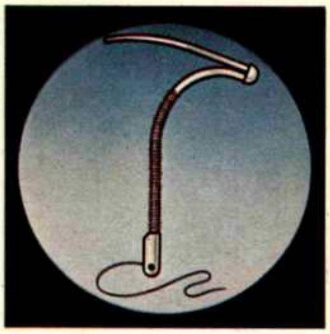

BY **SEARLE**

[1] Human Reproduction and Society, Faber, 1974
[2] Brit. Med. J., 1974, **3,** 447–450
[3] Data on files of Searle Laboratories
[4] IPPF booklet "Intrauterine Contraception"
[5] Am. J. Obstet. Gynecol. 1973, **117,** 602

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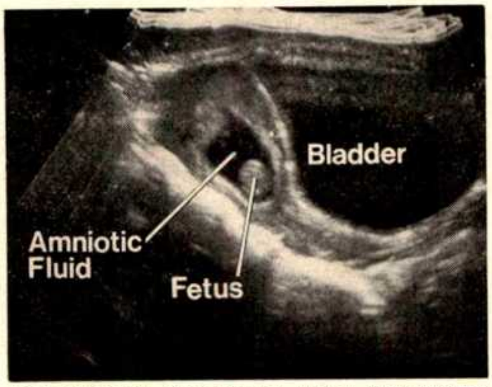

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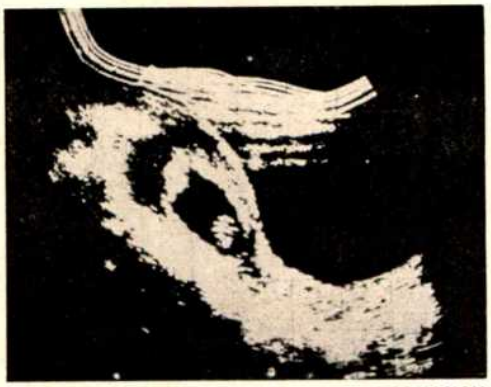
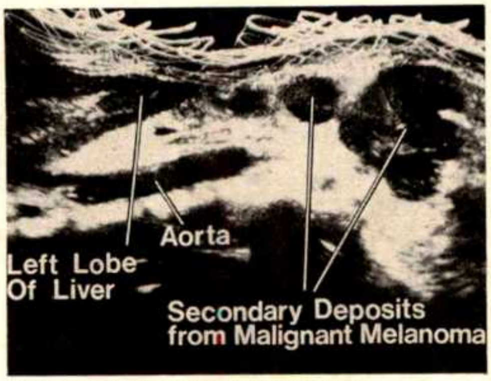
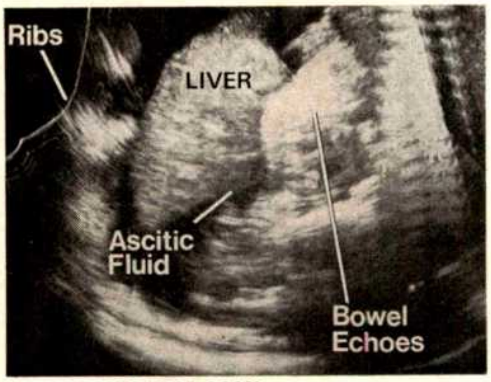


LONGITUDINAL SECTION OF EARLY PREGNANCY (NO GREYSCALING)



LONGITUDINAL SCAN OF ABDOMEN



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**References:** 1 *British Journal of Cancer* (1971), **25**, 270.
2 *British Medical Journal* (1973), **1**, 13.

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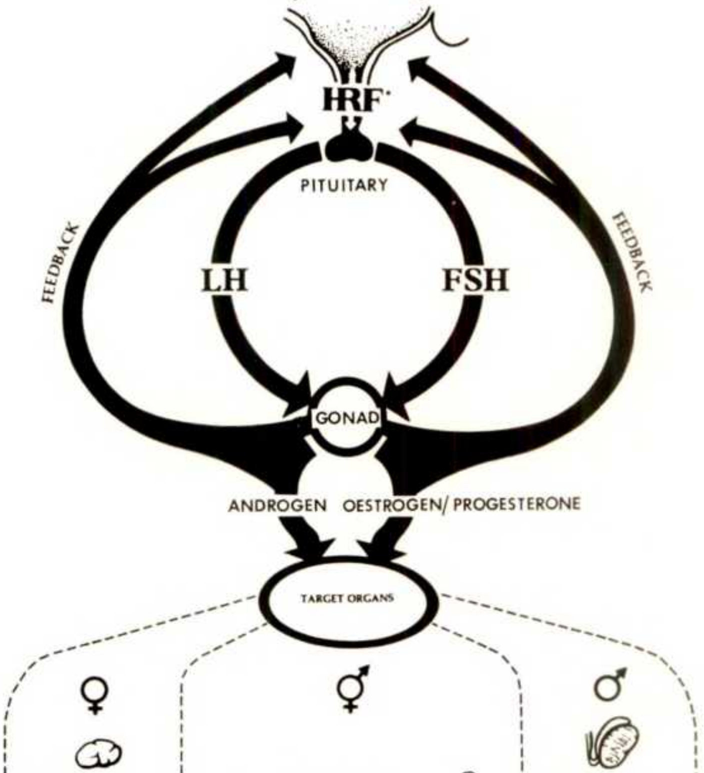
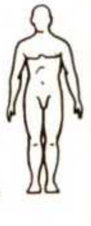


**Female**
Forbes-Albright syndrome
Sheehan's syndrome
Stein-Leventhal syndrome
Hypothalamic organic
  lesions
Hypogonadotrophic
    hypogonadism
  Primary amenorrhoea
  Secondary amenorrhoea
  —without galactorrhoea
    anorexia nervosa
    iatrogenic
    idiopathic
    psychogenic
    post-traumatic
    postpartum
    with galactorrhoea
    iatrogenic
    idiopathic
    non-postpartum
    postpartum

**Male and Female**
Acromegaly
Cushing's syndrome
Prader-Willi syndrome
Simmonds' syndrome
Hypophysectomy
Pituitary ablation
Hypothalamic disorders
  Tumours
  Congenital lesions
    Kallmann's syndrome
Functional disorders
Panhypopituitarism
  Primary
  Secondary
    idiopathic or other
Hypogonadotrophic
  hypopituitarism
  Adult
  Pubertal



**Male**
Hypothalamic traumatism
Frohlich's syndrome
Klinefelter's syndrome
Hypogonadotrophic
  hypogonadism
  Primary
    Cryptorchism
  Secondary (with or
    without oligospermia
    or azoospermia)
    iatrogenic
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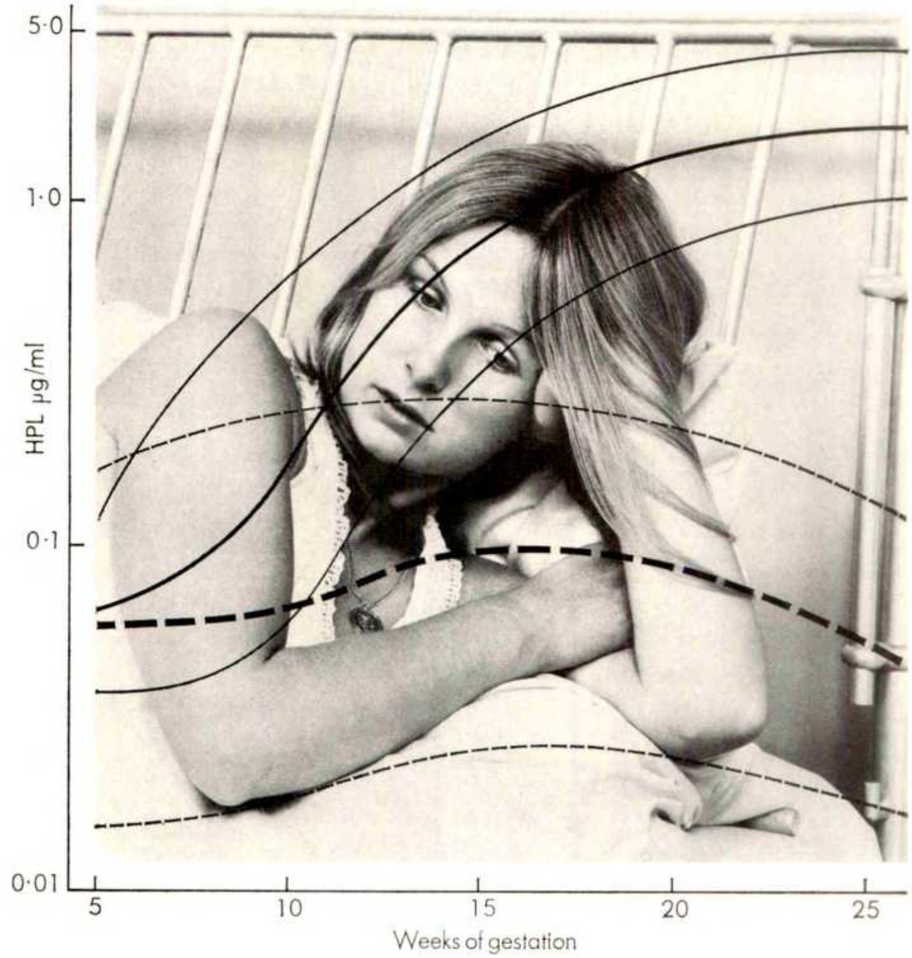

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**Reference** Brit Med J, **3**, 799-801, 1972.

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n m str al yc sds

e en ual cy le i s

ru c l

a c

Commencement of cycle

| Dosage | 26 | 27 | 28 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Menorrhagia/hypermenorrhoea**<br>1 tablet Primolut N b.d. or t.d.s. from the 19th-26th day of the cycle. | | | | | | | | | | | | | | | | | | | | | | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | | |
| **Dysmenorrhoea**<br>1 tablet Primolut N t.d.s. for 20 days, starting on the 5th day of the cycle. Treatment should be maintained for three or four cycles. | | | | | | | | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | ●●● | | | | |
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VOL. 82 No. 6 NEW SERIES JUNE 1975

# UTERINE ACTIVITY IN INDUCED LABOUR

BY
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*Department of Obstetrics and Gynaecology*
*St. Mary's Hospital Medical School, Praed Street, London W2*

## Summary

A study was made of the characteristics of oxytocin-induced labour. Twelve patients with, and eight without membrane rupture were closely matched for factors likely to influence the character and duration of labour. In both groups uterine activity increased until a stable state was achieved following which there was little alteration until delivery. The duration of labour was shorter in the group with ruptured membranes as compared with intact membranes although, paradoxically, the uterine activity tended to be greater when the membranes were intact.

EARLY reports from workers such as Keetel *et al.* (1940) studying spontaneous labour suggested that rupture of the membranes had no effect upon the duration of labour. Alvarez *et al.* (1961) studying labour induced with oxytocin, reported that amniotomy halved the time required to dilate the cervix from 4 to 10 cm. Friedman and Sachtleben (1963), studying spontaneous labour, hotly disputed this and stated that in cases of dysfunctional labour, amniotomy could slow labour. The 1973 Latin American Collaborative Study (Schwartz, 1973), specially designed to settle the issue, reported 182 normal spontaneous labours in which half the patients had an amniotomy at 4 cm. and the remainder had their membranes left intact. The mean durations of labour were 7 hours 35 minutes and 8 hours 17 minutes respectively, but this difference was not statistically significant.

Induction of labour by oxytocin infusion is usually combined with amniotomy and in many centres such as the National Maternity Hospital, Dublin (O'Driscoll *et al.*, 1973), Queen Charlotte's Maternity Hospital, London (Boyd, 1973), and our own Unit, it is carried out in 25 per cent or more of all pregnancies. Uterine stimulation by oxytocin is not without danger since hyperstimulation commonly produces transient fetal bradycardia and may occasionally cause uterine rupture (Daw, 1973). Amniotomy also is associated with a number of disadvantages such as amnionitis, fetal infection and prolapse of the umbilical cord (Gabert and Stenchever, 1973); and an increased variability of the fetal heart rate pattern with uterine contractions, whatever the significance of this may be (Gabert and Stenchever, 1973; Caldeyro-Barcia *et al.*, 1972).

In order to justify the modern policy of frequent induction it is essential that uterine activity in oxytocin induced labour should be characterized and that the effect of membrane rupture on the duration of labour should be finally settled.

A study was therefore designed to establish the pattern of uterine activity when labour is induced with oxytocin and to determine the influence of membrane rupture on the duration of labour. The effects on the fetal circulation will be reported in a separate paper.

## PATIENTS AND METHODS

Twelve patients were studied with ruptured membranes and eight with intact membranes. In order to minimize interfering factors, patients were only selected for the study if induction was planned for postmaturity, social reasons or some minor medical indications after an otherwise uneventful pregnancy. The reasons for induction are listed in Table I, which also compares some clinical features of the patients in the two groups. Initial assessment of the cervix was made using a modified Bishop score (Klopper *et al.*, 1969): In this system a hard cervix scored −1 while a soft cervix scored +1; a long cervix scored −1, a partly effaced cervix +1, and a fully effaced cervix +2; a closed cervix scored −1, cervical dilatation up to 1·5 cm. scored +1 and a greater dilatation +2. None of the patients had meconium stained amniotic fluid at amnioscopy during the initial vaginal examination. The progress of labour was followed by two of us (P.S. and D.L.) by 4-hourly vaginal examinations. Random selection was achieved in 17 patients by alternately rupturing or keeping membranes intact. In three patients the membranes ruptured accidentally during the insertion of the catheter used to record intrauterine pressure. Subsequent analysis showed that the uterine action in these patients was indistinguishable from that in patients who had had their membranes ruptured intentionally and they were therefore included in this group.

TABLE I

*Clinical features of the patients studied*

| Parameter | Unruptured membranes (8 patients) | Ruptured membranes (12 patients) |
|---|---|---|
| Number of days past estimated date of delivery Mean±SD | 7·6±9·3 | 5·1±6·1 |
| Maternal age Mean±SD | 27·9±4 2 | 25·3±5·9 |
| Dilatation of cervix at induction (cm.) Mean±SD | 2·6±0·9 | 2·8±1·0 |
| Head descent at induction (1/5ths) Mean±SD | 3·4±0·7 | 2·8±0·7 |
| Bishop score at induction Mean±SD | 3·8±0·9 | 4·2±0·9 |
| Method of delivery: | | |
| Forceps | 1 | 3 |
| Vacuum extraction | 1 | 1 |
| Apgar scores: 1 minute | 8·1±1·2 | 8·6±0·9 |
| 10 minutes | 9·8±0·4 | 9·8±0·4 |
| Parity 0 | 4 | 5 |
| 1+ | 5 | 8 |
| Reason for induction: | | |
| Postmature elective | 4 | 7 |
| Very mild pre-eclampsia | 3 | 1 |
| Small antepartum haemorrhage | 1 | |
| Minimal medical indication | | 4 |
| No. of patients who had epidural analgesia during labour | 5 | 6 |

No significant difference between the two groups as measured by $X^2$ or student's "t" test.

Oxytocin, at a concentration ranging from 2 to 32 mU./minute, was administered by infusion pump (Rocket of London) or IVAC drip counter. It was commenced at 2 mU./minute and increased by 2 mU./minute every 15 minutes until there were at least three contractions every 10 minutes.

Intrauterine pressure was recorded by means of an open-ended catheter (Hewlett Packard) filled with sterile distilled water and placed extra-amniotically above the fetal head as described by Csapo (1970). The catheter was connected to a pressure transducer which operated in conjunction with either the Hewlett Packard 8020A or the Sonicaid FM2 to provide a permanent record of intrauterine pressure. The parameters of uterine action are shown in Figure 1 and are according to Csapo and Savage (1970).

The "duration" of each contraction was the interval between the onset of the contraction (an abrupt rise of pressure from baseline) and the offset (a point of maximum inflection as the pressure returned to baseline; the "offset pressure" was usually about 5 mm. Hg. above the baseline) and this gave the best estimate of the time during which there was a significant rise in intrauterine pressure. "Active pressure"

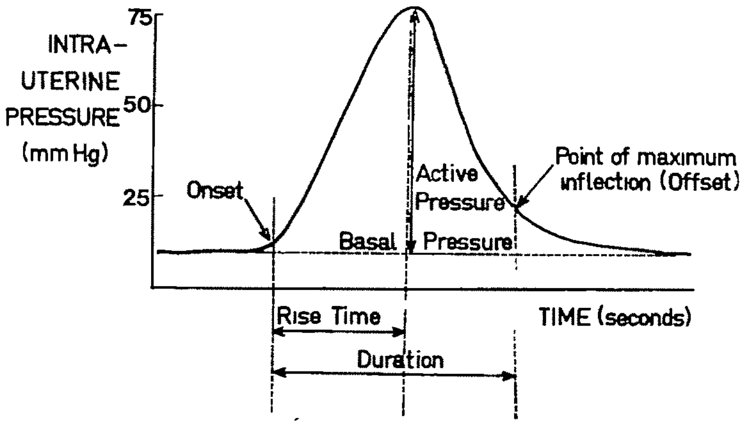

FIG. 1
The components of a uterine contraction (Csapo, 1970).

was defined as the peak pressure less the basal pressure for each contraction. "Basal pressure" or "tone" was not specifically analyzed because of the difficulty in a series of patients of maintaining the transducer at the same level relative to the patient on every occasion, but in all cases the baseline was within the normal limits of 10 to 15 mm. Hg. The "rise time" was the time taken to reach the peak pressure from the baseline. From these parameters, two further values were determined.

The ratio of active pressure to rise time (Ap/Tr) described the slope of the leading edge of the contraction, and indicated the rate at which intrauterine pressure is generated. "Activity units" were derived for each contraction by multiplying the active pressure by the duration of the contraction. For statistical purposes, activity units (as shown in Figs. 2 and 3, and Tables II and III) were summated over half-hour periods. All the other parameters of uterine action were expressed as mean values per half hour.

Although the exact relationship between intrauterine pressure and the energy output of the uterus during labour is not known, uterine pressure recordings remain the most objective means of assessing "work" done (Hon and Paul, 1973). The area under the contraction curve is known to give a more constant value than the Montevideo Unit (peak pressure and frequency) (Csapo, 1970; Krapohl *et al.*, 1965; Hon and Paul, 1973). It more closely parallels the effect of intrauterine pressure on placental blood flow, which is reduced steadily as the pressure rises until cessation occurs when the intrauterine pressure reaches 30 mm. Hg. (Towell, 1966), because it incorporates the duration over which the reduced flow occurs.

## RESULTS

*Uterine activity (see Figs. 2 and 3 and Tables II and III)*

Figures 2 and 3 show that uterine activity stabilized after a mean period of two hours in the sixteen patients with Bishop scores of 4 and 5, and after a mean period of 2·5 hours in the four patients with Bishop scores of 2 and 3. Figure 2 shows that in patients with a high Bishop score uterine activity stabilized by a mean time of one hour before the oxytocin infusion rate was also stabilized. In the low Bishop score group, shown in Figure 3, oxytocin was given in increasing dosage throughout labour.

These results suggest that there was a tendency to increase the oxytocin infusion rate for some time after the activity of the uterus had stabilized. This tendency is demonstrated more clearly by activity units than by active pressure or frequency of contractions. The rise in the Ap/Tr index at the onset of induction mirrors the rise in active pressure but the duration and rise time of contractions show no significant alteration throughout labour. When the individual results of

TABLE II

*Oxytocin dosage and uterine activity in 16 patients with a high Bishop score (4 or 5) at the time of induction (see Fig. 2)*

| | Half-hour periods after onset of induction | | | | | | Stable phase | Last half hour of stable phase |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | | |
| Oxytocin (mU./minute) Mean±SD | 4·2±2·8 | 6·9±3·0 | 9·4±3·8 | 11·3±4·3 | 12·9±4·9 | 13·4±5·7 | 12·7±5·4 | 13·8±6·0 |
| Activity units (×10³) Mean±SD | 9 8±6·9 | 15·1±9·1 | 19·1±7·5 | 25·1±10·2 | 25·0±10·1 | 23·9±7·5 | 26·5±7·8 | 27·5±9·6 |
| No. of contractions per half-hour Mean±SD | 6·5±4·4 | 8·9±3·4 | 9·5±2·6 | 11·7±1·7 | 12·3±2·2 | 12·1±2·3 | 12·6±1 7 | 13·3±2·6 |
| Active pressure (mm. Hg.) Mean±SD | 24·0±10·2 | 28·1±11·9 | 34·9±10·8 | 36·8±12·3 | 35·1±9·6 | 39·4±10·8 | 37·2±9·4 | 36·9±9·7 |

TABLE III

*Oxytocin dosage and uterine activity in four patients with a low Bishop score (2 or 3) at the time of induction (see Fig. 3)*

| | Half hour periods after the onset of induction | | | | | | | | Stable phase | Last half hour of stable phase |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | | |
| Oxytocin (mU./minute) | 4·5±2·5 | 7·0±2·0 | 10·0±4·3 | 12·5±5·7 | 13·0±6·2 | 14·0±6·9 | 14·5±7·2 | 15·5±6·6 | 16·4±9·1 | 17·0±10·0 |
| Activity units (×10³) Mean±SD | 3·6±1·1 | 5·0±3·8 | 12·8±10·7 | 17·9±10·7 | 23·3±13·7 | 22·9±12·0 | 27·3±11·9 | 24·5±9·7 | 26 5±8·8 | 25·3±13·3 |
| No. of contractions per half hour Mean±SD | 1·75±0·9 | 4·8±4·1 | 7·3±5·9 | 9·0±4·0 | 11·3±2·2 | 9·5±1·3 | 10·8±1·3 | 11·0±1·8 | 11·3±1·6 | 12·2±3·2 |
| Active pressure (mm. Hg.) Mean±SD | 22·4±5·0 | 25·9±2·9 | 30·0±7·8 | 33·1±14·1 | 34·8±12·2 | 38·5±12·2 | 41·1±13·6 | 39·3±9·8 | 38 3±7·2 | 33·1±13·3 |

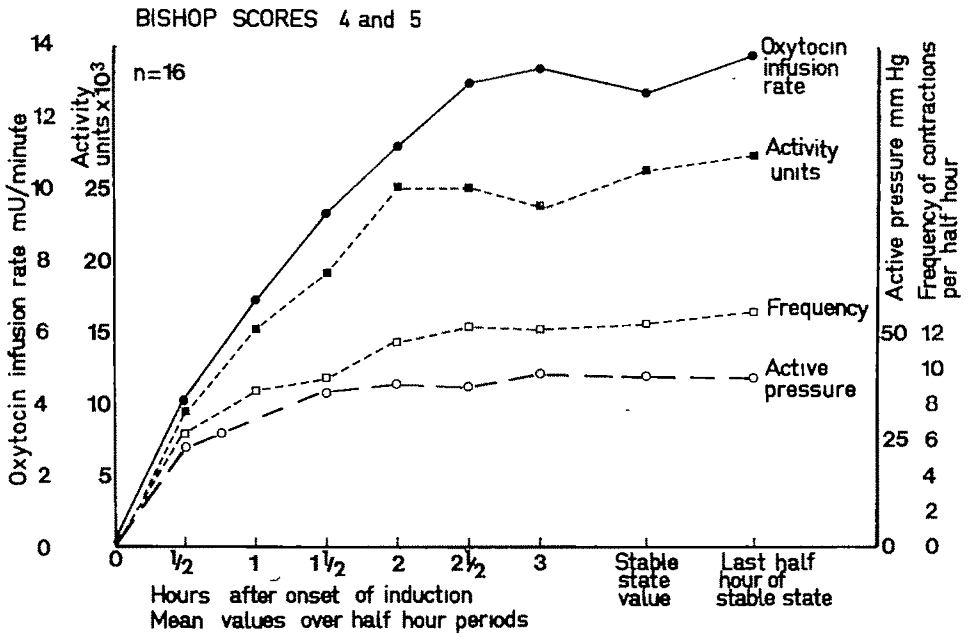

FIG. 2

Parameters of uterine activity. Mean values at various stages of labour in 16 patients with high Bishop scores at the time of induction (see Table II).

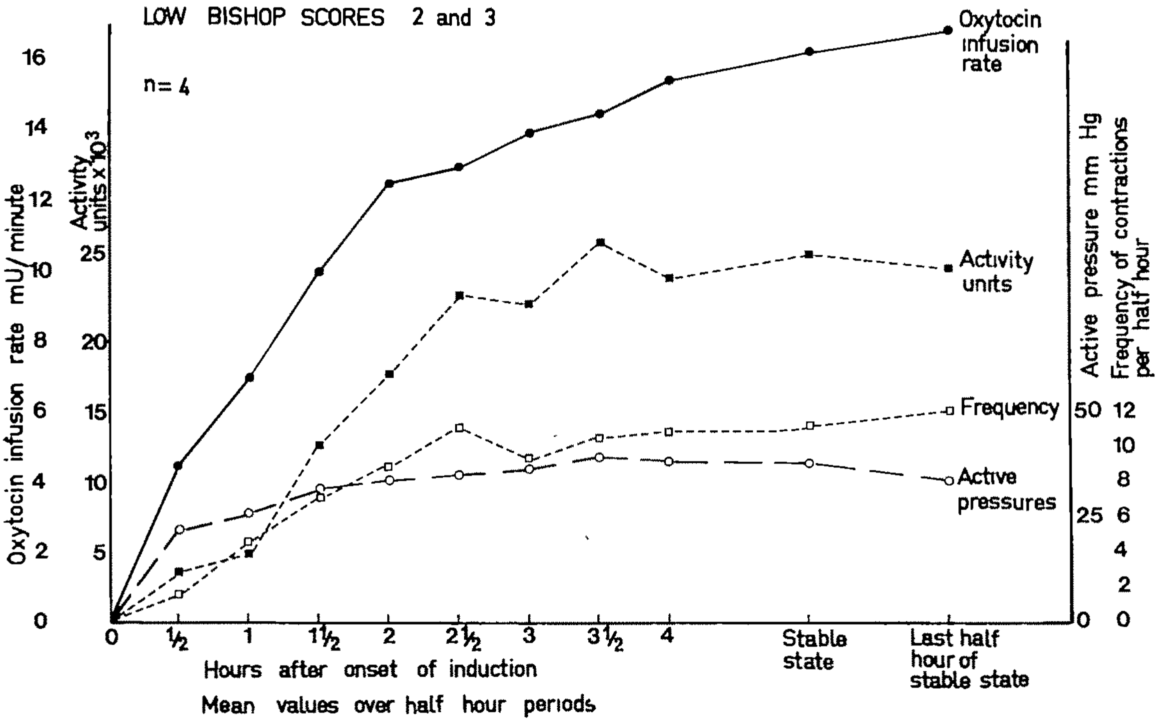

FIG. 3

Parameters of uterine activity. Mean values at various stages of labour in four patients with low Bishop scores (2 and 3) at the time of induction.

induced patients were re-examined, it was found that in 12 patients, stabilization of uterine activity occurred while oxytocin was still being increased. In eight patients, oxytocin infusion was stabilized at the same time as uterine activity stabilized.

One may therefore conveniently consider labour in three parts. The "incremental phase" lasts from the time the uterus starts to contract to the time that stabilization of activity occurs. The "stable phase" is the time during which there is only a small net change in uterine activity. The stable phase was present at both high and low Bishop scores. There was a small net increase in activity in 11 patients, a small decrease in eight, and in one patient no change over this phase. The "final phase" corresponds with the second stage of labour which is complicated by pushing when it did not prove possible to determine the intrinsic uterine activity from the intrauterine pressure trace alone.

The duration of the incremental phase of uterine activity in the two groups reflects the increased sensitivity of the uterus to oxytocin when the cervix is favourable; in other words the higher the Bishop score, the greater the sensitivity, the shorter the incremental phase and the shorter the duration of labour (the mean duration of labour in patients with a Bishop score of 4 or 5 was 6·25±2·6 (SD) hours, and in patients with a Bishop score of 2 or 3 it was 10·25±4·2 hours). However, the incremental phase appeared to be least affected by the Bishop score and was never longer than two hours if the Bishop score was 4 or 5 or 2·5 hours if the Bishop score was 2 or 3. Figure 4 shows that the duration of the incremental phase was not affected by the state of the membranes.

Comparison of activity units during the stable phase suggests that uterine activity tends to be greater when the membranes are intact rather than ruptured. Mean activity units per half hour in the ruptured group were 24·33±8·76 (SD) $\times 10^3$ and in the unruptured group 29·74±4·70 $\times 10^3$ ($t = 1{\cdot}79$; $p < 0{\cdot}05$). Table VI shows that

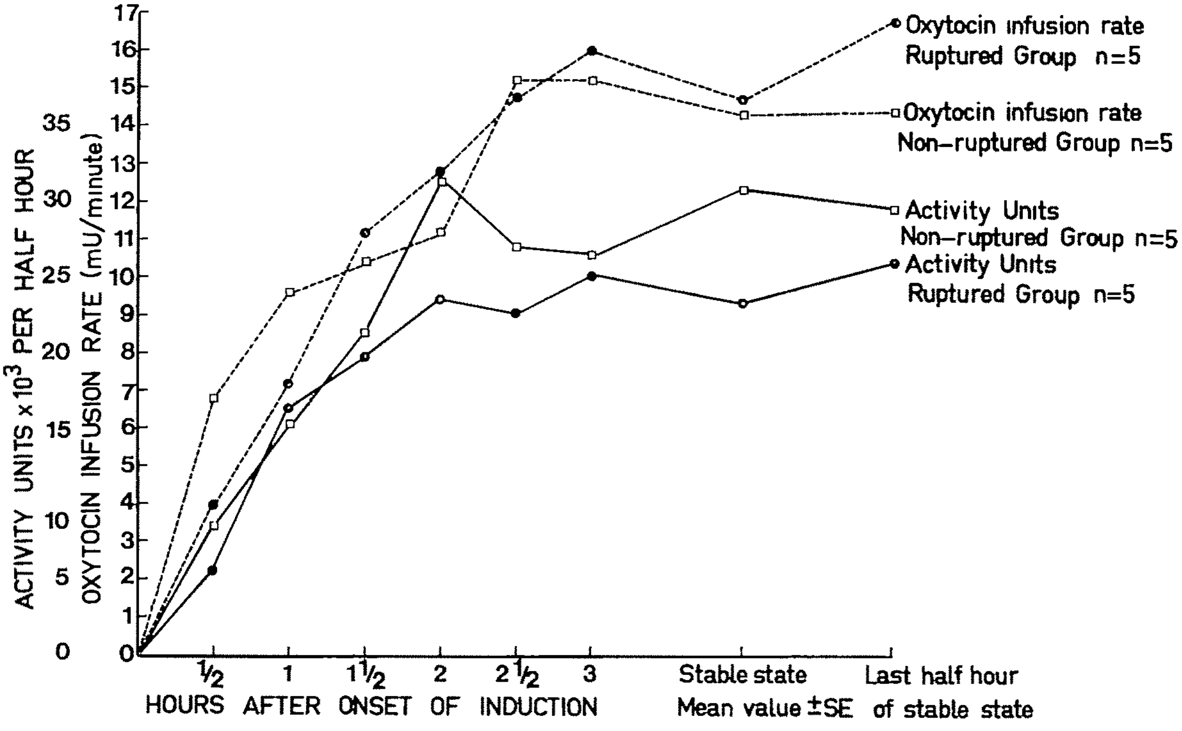

FIG. 4

The onset of the stable phase in patients with ruptured membranes and in patients with intact membranes—all patients with an initial Bishop score of 4.

TABLE IV

*Stable state values in patients with intact and ruptured membranes*

| Parameter | | Patients with intact membranes | Ruptured membranes | "t" or $\chi^2$ | Significance of difference |
|---|---|---|---|---|---|
| Activity units per half hour ($\times 10^3$) | Mean±SD | 29 7±4·7 | 24·3±8·8 | 1·79 | p<0·05 |
| Active pressure (mean Hg.) | | 42·2±4·8 | 34·0±9·5 | 2·55 | p<0·01 |
| Duration of contraction (seconds) | Mean±SD | 59·7±12·7 | 57·1±7·2 | 0·52 | p<0·7 |
| No. of contractions per half hour | Mean±SD | 12·0±1·7 | 12·1±1·9 | 0·1 | p<0·95 |
| Rise time (seconds) | Mean±SD | 25·4±6·5 | 24·2±5·8 | 0·41 | p<0·2 |
| Ap/tr (profile) | Mean±SD | 1·82±0·58 | 1·49±0·41 | 1·4 | p<0·2 |
| Oxytocin (mU./minute) | Mean±SD | 17·1±4·9 | 10·3±5·9 | 2·80 | p<0·02 |

when the membranes are intact this increase can be ascribed entirely to the development of higher intrauterine pressures (p<0·01). Duration, frequency and Ap/Tr of contractions are not significantly different in the two groups. A possible explanation for the higher pressures recorded when the membranes were intact is that the mean oxytocin infusion rate of 17·1 mU./minute for the stable state in the patients with intact membranes was higher than the mean rate of 10·3 mU./minute for the stable state in the patients with ruptured membranes (p<0·02). This difference in oxytocin infusion rate was predominantly in the low Bishop score group; it was not evident where the cervix was favourable (Fig. 4). The tendency to use more oxytocin when the membranes were intact was probably due to the efforts of the clinician to achieve the same frequency and duration of contractions in patients with intact as with ruptured membranes.

### *Duration of labour*

The mean duration of labour in the group of patients with ruptured membranes was 5·54±2·53 (SD) hours, and this was significantly shorter than 9·31±3·70 hours for the group of patients with intact membranes (t = 2·98; p<0·005). Within the groups the duration of labour was shown to relate to parity, primiparae taking a mean 9·1±3·75 (SD) hours, and multiparae 6·0±3·08 hours (p<0·005). The duration of labour was directly related to the Bishop score, but as with parity this parameter was equally distributed between the two groups of patients.

There was also an inverse relationship between the Bishop score and the rate of oxytocin infusion once this had been stabilized. In other words, the higher the Bishop score, the lower the amount of oxytocin required to maintain uterine activity at a clinically acceptable level.

In the five multiparae whose membranes were allowed to rupture spontaneously, this occurred at a mean of 24·4±22·15 (SD) minutes before delivery and the mean duration of the second stage was 22·4±20·3 minutes. In three primiparae, spontaneous membrane rupture occurred at a mean of 165±34·8 minutes before delivery and the mean duration of the second stage was 38·3±18·5 minutes.

## DISCUSSION

This study has characterized the pattern of uterine activity in uncomplicated, induced labour. Initially there was an incremental phase during which uterine activity steadily increased until it reached a plateau in the stable phase. The duration of the incremental phase varied with the state of the cervix (although it was never longer than 2·5 hours) and it was not influenced by the state of the membranes. Once the stable state had been reached, uterine activity did not show a great deal of variation despite attempts to increase it by increasing the oxytocin infusion rate.

These observations suggest that there is little point in attempting to increase uterine activity in the stable phase by extra stimulation; indeed this may produce incoordinate uterine action. This hypothesis is supported by a recent comparison of labour induced with oxytocin and labour induced with prostaglandins (Kelly *et al.*, 1973). Despite significantly higher levels of baseline tone, mean amplitude and frequency of contractions in the oxytocin group, this group had a longer induction to delivery interval, associated with a significantly higher incidence of incoordinate uterine activity.

It should be stressed that our study was not specifically designed to investigate the pattern of uterine activity that develops during labour and a prospective study will have to be done to confirm beyond doubt the pattern we have described. Equipment for quantifying uterine activity is being developed so that it may prove possible to detect the onset of the stable phase. This should provide a means of detecting optimal uterine activity at an oxytocin dosage which is minimal and therefore well within a margin of safety. This is particularly important in view of the increasing sensitivity of the uterus as labour progresses (Sica-Blanco *et al.*, 1961; Krapohl *et al.*, 1965).

Our findings also support the clinical impression that rupturing the membranes shortens induced labour, as stated by Alvarez *et al.* (1961). It is likely that previous disagreements in the literature are due to the fact that insufficient attention has been given to the difference between induced and spontaneous labour. Workers who have demonstrated the speeding effect of amniotomy were studying oxytocin-induced labour, and those who have failed to demonstrate the effect were studying spontaneous labour. Normal spontaneous uterine activity occurs under optimal conditions, and it is therefore reasonable to expect that measures such as amniotomy or even oxytocin infusion will fail to expedite a labour which is already occurring at maximum speed.

Since induced labour is by definition taking place when the uterus is incompletely prepared for contraction, it follows that oxytocin infusion will produce more effective activity; and it seems that amniotomy can also speed labour by increasing the efficiency of those contractions which do occur.

Our findings show that the oxytocin-driven uterus is more efficient when the membranes are ruptured, activity per unit time being less in addition to the shorter labour than when the membranes are intact. The mechanism by which membrane rupture improves the efficiency of uterine action is not clear. It is possible that the effect is purely mechanical. With a closed fluid system pressure is evenly distributed throughout the uterine cavity. Once a leak has developed a greater component of uterine wall tension acts through the longitudinal axis of the fetus. This results both in an increased force dilating the cervix and a decrease in the production of intrauterine pressure (Smythe, 1973). This extra force may be necessary in induced labour because the cervix is not as completely prepared as in spontaneous labour.

## ACKNOWLEDGEMENTS

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# THE UTERINE AND CARDIOVASCULAR EFFECTS OF SALBUTAMOL AND PRACTOLOL DURING LABOUR

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## Summary

Intravenous salbutamol, a $\beta$-adrenoceptor stimulant, given to nine patients in normal labour, with continuous monitoring of uterine activity and of the maternal and fetal cardiovascular systems, was shown to decrease uterine activity significantly; maternal and fetal heart rates were significantly increased, and maternal systolic and diastolic arterial pressures were significantly decreased during the infusion, although no treatment had to be discontinued because of these effects. Apart from worsening of low back pain during the infusion in one patient, subjective side-effects were trivial. With the salbutamol infusion continued at an effective maintenance rate, the cardioselective $\beta$-adrenoceptor blocking drug, practolol, given intravenously, reduced the maternal heart rate (although not significantly) but it did not alter the fetal heart rate; it also appeared to interfere transiently with the inhibiting action of salbutamol on uterine activity, but cervical dilatation was arrested until the salbutamol infusion was discontinued. At least in five patients, labour remained suppressed until oxytocin was infused intravenously.

VARIOUS drugs which inhibit uterine activity have been investigated in the attempted arrest of premature labour, including intravenous ethyl alcohol (Fuchs *et al.*, 1967; Zlatnik and Fuchs, 1972), diazoxide (Landesman *et al.*, 1969), and such $\beta$-sympathomimetic drugs as isoxsuprine (Hendricks *et al.*, 1961; Allen *et al.*, 1965), ritodrine (Coutinho *et al.*, 1969; Beveridge and Hood, 1970; Nochimson *et al.*, 1974; Wesselius-De Casparis *et al.*, 1971) and orciprenaline (Baillie *et al.*, 1970; Tyack *et al.*, 1971). Most of them have side effects which limit their use, such as maternal tachycardia, palpitations and hypotension when the $\beta$-sympathomimetic drugs are used (Drug and Therapeutics Bulletin, 1973), the myocardial effects being due to stimulation of $\beta_1$-adrenoceptors in the heart.

Recently, Liggins and Vaughan (1973) demonstrated that intravenous infusion of salbutamol, a non-catechol $\beta$-adrenergic stimulant with selective action on $\beta_2$-receptors (Paterson *et al.*, 1971), inhibited uterine activity in premature labour and postponed delivery in more than 85 per cent of cases.

The present study was undertaken to assess the maternal and fetal effects of the intravenous infusion of salbutamol during normal labour and to gain experience of the use of practolol, a cardioselective β-adrenoceptor blocking drug (Dunlop and Shanks, 1968), in the control of cardiovascular side effects.

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## METHODS

Nine normal patients (8 primigravidae) in early labour at term, with uterine contractions occurring at least every ten minutes, were studied with the approval of the regional ethical committee. Cervical effacement and dilatation were assessed and the membranes, if still intact, were ruptured. Intrauterine pressure and fetal heart rate were recorded continuously, using a Corometrics FMS-102 Monitor with a transcervical catheter and fetal scalp electrode; composite uterine activity was calculated in Alexandria units (El-Sahwi *et al.*, 1967). Maternal heart rate was recorded every five minutes with an electrocardiogram (Lead I) by measuring the time taken for five complete cardiac cycles, and was also continuously monitored on an oscilloscope; maternal blood pressure was recorded every five minutes by sphygmomanometry. Baseline recordings (uterine contractions, fetal and maternal heart rates, and blood pressure) were made for 30 minutes prior to infusion of salbutamol.

Salbutamol, 5·0 mg. in 500 ml. of 5·0 per cent dextrose solution, was then infused through a forearm vein using an "Ivac" infusion pump. In eight patients, an initial rate of 10 μg. of salbutamol per minute was increased every 5 or 10 minutes by increments of 10 μg. per minute until either uterine activity ceased or the maternal heart rate exceeded 140 beats per minute. The infusion rate at which either of these events occurred was then maintained for 30 or 60 minutes, following which (with the infusion continuing at the maintenance rate) practolol, 0·4 mg. per kg., was given intravenously over a three-minute period to seven of the patients, in whom uterine pressure, fetal heart rate, maternal heart rate and blood pressure were recorded for a further 30 minutes; similarly, one patient received propranolol, 2·0 mg. intravenously, instead of practolol; the remaining patient had a constant rate infusion of 30 μg. of salbutamol per minute (instead of increasing doses) before receiving practolol (as above).

In all nine patients, the salbutamol infusion was discontinued 30 minutes after injection of the β-adrenoceptor blocking drug, and labour was allowed to continue or, when necessary, oxytocin was infused intravenously to re-establish effective uterine action. Cord blood was obtained from each baby for estimation of blood practolol level, and the Apgar score was recorded at one and five minutes after birth.

## RESULTS

The main features of each patient studied, including the pre-salbutamol (baseline) findings, the treatment given and its effects on labour, are shown in Table I. The duration of labour before infusion of salbutamol was between 2 and 11 hours (mean 6·8), cervical dilatation varied from 2 to 5 cm., and in 8 of the 9 patients effacement was graded as at least 90 per cent.

### *Effects of increasing salbutamol dosage*

The effects of incremental increases in the salbutamol infusion rate from 10 to 30 μg. per minute in eight patients are summarized in Table II. Uterine action was profoundly affected by the salbutamol infusion, the strength and duration of contractions being significantly reduced by increasing concentrations of the drug although the basal uterine tone altered very little. The reduction in mean Alexandria units from a basal level of 277·0 (±65·3 SEM) to 19·0 (±7·7 SEM) after 30 μg. per minute showed a significantly linear dose response.

In patient C, although salbutamol reduced the strength and frequency of uterine contractions, it was associated with worsening of lower back pain (present before the infusion) and with tachycardia, and practolol was therefore given before salbutamol was increased to 50 μg. per minute, at which rate the pain eased and the labour was interrupted until the infusion was discontinued. Because of the variation from protocol, this patient was not included in the further analysis.

In the other seven patients, the infusion rate was increased to 50 μg. per minute in two (A and G), to 40 μg. per minute in one (E), and to 30 μg. per minute in four before a substantial effect on uterine action was seen.

Mean maternal heart rate values rose from 99·9 (baseline) to 135·8 beats per minute, but, although individual rates occasionally fluctuated above 140 beats per minute, it was never neces-

TABLE I
*Details of patients*

| Patient | Gestational age (weeks) | Progress before salbutamol | | | Salbutamol infusion | | | | Progress after salbutamol | | | Type of delivery | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Labour (hours) | Cervix Effacement (per cent) | Cervix Dilatation (cm.) | Duration (hrs, mins) | Time to constant infusion (minutes) | Maximum dose (μg./mins) | Total dose (mg.) | Labour suppressed (Yes/no) | Time to resumed labour (hrs, mins) | Time to delivery (hrs, mins) | | |
| A | 41 | 6 | 100 | 4 | 1, 48 | 22 | 50 | 4·87 | No | 0 | 5, 35 | Normal | Propranolol |
| B | 40 | 7 | 90 | 4 | 1, 45 | 15 | 30 | 2·95 | Yes | 3, 45 | 7, 55 | Normal | Oxy |
| C | 40 | 4 | 100 | 2 | 1, 35 | 32 | 40 | 3·42 | Yes | 1, 40 | 7, 35 | Normal | Oxy |
| D | 39 | 9 | 100 | 2 | 1, 15 | 10 | 30 | 2·09 | Yes | 3, 45 | 9, 20 | Normal | Oxy |
| E | 40 | 10 | 100 | 2 | 1, 58 | 20 | 40 | 4·37 | Yes | 3, 12 | 10, 00 | Forceps | Oxy |
| F | 40 | 2 | 100 | 4 | 1, 00 | immediate | 33 | 1·98 | Yes | 1, 15 | 8, 15 | Normal | Oxy |
| G | 40 | 11 | 100 | 5 | 2, 10 | 28 | 50 | 5·90 | ? | 2, 06 | 12, 35 | Forceps | Oxy |
| H | 40 | 4 | 100 | 4 | 1, 42 | 11 | 30 | 2·89 | No | 0, 10 | 3, 10 | Forceps | |
| J | 39 | 6 | 50 | 2 | 2, 10 | 10 | 30 | 4·14 | ? | 4, 40 | 12, 50 | Vacuum | Oxy, Para 1 |

Oxy = Oxytocin (intravenous infusion) needed to re-establish effective uterine action.
Patient C excluded from complete analysis because practolol given before reaching salbutamol steady state.

TABLE II
*Effects of increasing infusion rates of salbutamol*

| | | Baseline Mean±SEM | Salbutamol infusion rate (μg. per minute) 10 Mean±SEM | 20 Mean±SEM | 30 Mean±SEM |
|---|---|---|---|---|---|
| *Uterine contractions* | Strength (mm. Hg.) | 45·0±5·3 | 37·8±5·7 | 26·1±4·4 | 10·4±3·4*† |
| | Duration (seconds) | 106·6±8·8 | 97·6±10·3 | 104·5±13·9 | 47·6±18·7*† |
| | Basal tone (mm. Hg) | 10·4±1·4 | 10·3±1·3 | 9·8±1·2 | 9·0±1·3 |
| | Alexandria units** | 277·0±65·3 | 129·4±28·1 | 80·5±25·7 | 19·0±7·7*† |
| *Maternal* | Heart rate (beats per minute) | 99·9±6·6 | 114·5±9·5 | 124·3±6·5 | 135·8±4·2* |
| | Systolic pressure (mm. Hg.) | 123·1±3·4 | 120·6±4·9 | 118·8±5·2 | 116·9±6·0 |
| | Diastolic pressure (mm. Hg.) | 82·5±3·3 | 77·5±3·5 | 73·1±2·8 | 68·1±4·2*† |
| *Fetal* | Heart rate (beats per minute) | 141·3±2·6 | 141·9±3·4 | 145·6±3·5 | 144·4±3·1 |

** Alexandria units = Number of contractions every 10 minutes × peak above resting intrauterine pressure (mm. Hg.) × duration of contractions (minutes) (El-Sahwi *et al.*, 1967).

* Significantly linear dose response.

† 30 μg. per minute value significantly different from 10 μg. per minute value at 5 per cent level using Student's "t" test.

sary to stop or reduce the infusion because of maternal tachycardia or palpitations. Mean blood pressure values fell, but only the fall in diastolic pressure was significant. The mean fetal heart rate remained relatively constant.

### *Effects of constant rate salbutamol infusion*

The effects of salbutamol infusion at a constant maintenance rate in the six patients who received this for 60 minutes prior to the injection of practolol are summarized in Table III.

The suppression of uterine activity was maintained through the constant infusion period, although the further reduction in mean Alexandria units from 40·2 (±17·4 SEM) to 23·8 (±8·9 SEM) did not reach statistical significance.

Both the maternal and fetal heart rates increased significantly during the constant rate infusion. The maternal systolic and diastolic arterial pressures were significantly lower at the end of infusion than during the baseline recordings, but they appeared to be lowest after 30 minutes infusion and then to increase again.

In Patient F who received salbutamol at 30 $\mu$g. per minute without initial dose ranging, uterine contractions were arrested immediately and the maternal heart rate never rose above 120 beats per minute.

The subjective side effects of salbutamol were inconsequential, although on direct questioning several patients admitted to being aware of their rapid heart rate.

### *Effects of intravenous practolol during constant salbutamol infusion*

The effects of practolol (7 patients) at 5 and 30 minutes after injection are summarized in Table IV.

Several patients complained of one or two painful uterine contractions immediately after the practolol injection but this subjective effect was transient. The strength of uterine contractions and the mean Alexandria units had already increased significantly five minutes after the practolol bolus, and by 30 minutes, although there was some reduction in mean contraction strength, the mean Alexandria units continued to be increased significantly because of more frequent contractions.

A fall in mean maternal heart rate by only ten per cent (from 139·3 to 126·4 beats per minute) five minutes after giving practolol indicates that significant β-blockade was not obtained, although the maternal systolic blood pressure had increased significantly (to mean±SEM values of 117·1 mm. Hg.±6·8) at this stage.

### *Effect of propranolol*

Unlike practolol, propranolol (Patient A) immediately reversed the effects of salbutamol and effective labour recommenced despite the continuation of salbutamol infusion. The uterine basal tone and the strength and duration of contractions were markedly increased, and the frequency of contractions was doubled within 30 minutes of the propranolol injection. Significant β-blockade was demonstrated within five minutes, by which time the maternal heart rate had fallen from 142 to 116 beats per minute (a reduction by 18 per cent), and was maintained to 30 minutes. The fetal heart rate fell immediately and even more dramatically, from 180 to 130 beats per minute (a 28 per cent reduction), but after 30 minutes it had risen to 165 beats per minute.

### *Progress after discontinuation of salbutamol infusion*

No patient showed any increase in cervical dilatation or effacement while salbutamol was infused. In five patients in whom labour subsequently seemed to remain completely suppressed (for up to 4 hours, 40 minutes) despite discontinuation of the salbutamol, and in two others in whom this effect was less certain, oxytocin was infused intravenously to re-establish effective uterine activity. Apart from the patient given propranolol, there was only one other (H) in whom it was apparent that the salbutamol infusion had not clearly suppressed labour.

In all cases, delivery and the third stage of labour were uncomplicated. The five minute Apgar score was never less than seven, and all the infants subsequently thrived and remained well. Practolol was not found in the cord blood of any of the infants at delivery.

## DISCUSSION

The results show that salbutamol is capable of inhibiting uterine activity during normal labour, mainly by reducing the strength and frequency

TABLE III

*Effects of constant rate salbutamol infusion for 60 minutes in six patients*

| | | Baseline | Duration of constant salbutamol infusion (minutes) | | |
|---|---|---|---|---|---|
| | | Mean±SEM | 0<br>Mean±SEM | 30<br>Mean±SEM | 60<br>Mean±SEM |
| *Uterine contractions* | Strength (mm. Hg.) | 47·0±6·6 | 17·3±1·9 | 14·3±3·2 | 14·2±4·8* |
| | Duration (seconds) | 107·2±12·0 | 84·7±6·0 | 58·2±16·6 | 59·2±21·3 |
| | Basal tone (mm. Hg.) | 10 5±1·7 | 9·3±1·9 | 7·0±1·0 | 8·0±2 0 |
| | Alexandria units | 301 7±83·6 | 40·2±17·4 | 28·7±11·8 | 23·8±8·9 |
| | Frequency** | 12·8±1·2 | — | 7·0±1·4* | 6·0±1·2* |
| *Maternal* | Heart rate (beats per minute) | 95·8±8·3 | 123·0±6·3 | 136·5±1·5 | 140·2±3·8*† |
| | Systolic pressure (mm. Hg.) | 125·0±4·3 | 119 2±5·2 | 106·7±4·9 | 110·8±6·9* |
| | Diastolic pressure (mm. Hg.) | 85·0±3·7 | 75·0±3·7 | 63 3±6·7 | 67·5±6·6* |
| *Fetal* | Heart rate (beats per minute) | 140·8±3·5 | 145 0±4·8 | 158·3±3·1 | 165·8±3·7*† |

** Frequency = number of contractions occurring in previous 30 minute period.
* Value significantly different from baseline values at 5 per cent using Student's "t" test.
† Value significantly different from 0 minute value at 5 per cent level using Student's "t" test.

TABLE IV

*Effects of intravenous practolol, 0·4 mg. per kg., in seven patients during constant salbutamol infusion*

| | | Period from practolol injection (minutes) | | |
|---|---|---|---|---|
| | | 0<br>Mean±SEM | 5<br>Mean±SEM | 30<br>Mean±SEM |
| *Uterine contractions* | Strength (mm. Hg.) | 11·0±4·4 | 22 4±3·5* | 18 2±2·3 |
| | Duration (seconds) | 42·9±17·0 | 81·3±9·0 | 85 3±13 9 |
| | Basal tone (mm. Hg.) | 8 3±1 6 | 8·6±1 1 | 7·2±1·3 |
| | Alexandria units | 13·9±6·1 | 57 9±14·8* | 45·2±11 3* |
| | Frequency** | 6·0±1·2 | | 8·2±0·8* |
| *Maternal* | Heart rate (beats per minute) | 139·3±4·9 | 126·4±3·8 | 133·3±3·5 |
| | Systolic pressure (mm. Hg.) | 107·1±7·4 | 117 1±6·8* | 111 4±7·0 |
| | Diastolic pressure (mm. Hg.) | 63·6±6·1 | 70·0±6·9 | 70·0±5·7 |
| *Fetal* | Heart rate (beats per minute) | 157·1±4·3 | 157·9±2 9 | 159·2±3·0 |

** Frequency = number of contractions occurring in previous 30 minute period.
* Value significantly different from 0 minute value at 5 per cent level using Student's "t" test.

of uterine contractions, with more variable effect on their duration and apparently none on basal tone. In all our patients, uterine activity was inhibited profoundly throughout the salbutamol infusion, except after the administration of propranolol to one patient. Even when complete suppression of uterine activity was not accomplished, there was no progression of cervical effacement or dilatation despite the fact that the membranes were ruptured. Tachyphylaxis was not observed during the course of the infusion.

Although, in man, salbutamol does not cause tachycardia when effective bronchodilator doses are taken by aerosol or orally, in this study intravenous infusion of salbutamol in concentrations necessary to inhibit uterine activity caused a 40 per cent mean increase in heart rate which is comparable to results obtained in acute studies using ritodrine hydrochloride (Landesman *et al.*, 1971; Barden, 1972; Nochimson *et al.*, 1974). Warrell *et al.* (1970) have shown that the effects of salbutamol on the cardiovascular system, though less than those of isoprenaline, were not negligible and that infusion rates of between 0·24 and 0·3125 µg. per kg. per minute were associated with tachycardia and/or lowering of the diastolic pressure. In the present study both mean systolic and diastolic pressures were significantly lower after salbutamol than before it, whereas in a previous study of intravenous salbutamol in premature labour Liggins and Vaughan (1973) noted a consistent fall only in the diastolic pressure.

The fetal heart rates also increased, although much less rapidly, suggesting that salbutamol crosses the placenta into the fetal circulation but that there is some delay in reaching significant levels. The mean fetal heart rate increase of about 20 beats per minute after 90 minutes of salbutamol infusion is comparable with the findings of Liggins and Vaughan (1973).

Of some concern is the fact that both maternal and fetal heart rates increased significantly during the constant infusion period. However, as the elimination half-life for radioactively labelled salbutamol after oral administration or inhalation ranges from 1·7 to 7·1 hours (Walker *et al.*, 1972), steady state plasma salbutamol levels would not have been expected for at least 6 to 8 hours from the start of the constant infusion. Therefore, because effective salbutamol concentrations were achieved before the constant rate infusion began, higher concentrations than necessary must have been reached subsequently. Knowledge of the minimum plasma salbutamol concentration at which effective uterine inhibition occurs and of the drug's elimination half-life when administered intravenously should enable regimens to be formulated, using an initial bolus injection followed by a constant infusion of lower concentration to achieve therapeutic efficacy rapidly and to reduce toxicity.

Practolol was much less effective than propranolol in reversing the chronotropic effects of salbutamol. Similar findings with isoprenaline have been reported by Brick *et al.* (1968) who suggested that, whereas practolol and propranolol both block the component of the isoprenaline tachycardia that is due to direct stimulation of the sino-atrial node, only propranolol affects that due to reflex vagal inhibition from lowering of arterial pressure. Nevertheless, practolol caused considerable reduction of heart rate in some of our patients, in one of whom this effect allowed the concentration of salbutamol to be increased to a level at which uterine suppression occurred. Although it has been reported that practolol does not reverse the inhibiting effects of isoprenaline on the rat uterus (Wasserman and Levy, 1972), it did appear to have a transient effect on the uterine β-receptors in this study but, in contrast to propranolol, this was never sufficient to re-establish labour. The unchanged fetal heart rates and the absence of detectable practolol in the cord blood at delivery suggest that this drug does not cross the placenta into the fetus—measurable amounts of practolol would still be present in maternal blood ten hours after the intravenous injection of 20 to 30 mg. (Carruthers *et al.*, 1974). In contrast, propranolol does appear to cross the placenta. Thus, although practolol is not an ideal drug in this situation, it may sometimes be of value in allowing uterine inhibition by β-adrenoceptor stimulants to be continued after the occurrence of maternal cardiovascular side-effects.

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# THE EFFECTS ON PLATELET AGGREGATION OF ORAL PROSTAGLANDIN $E_2$ USED FOR THE INDUCTION OF LABOUR

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## Summary

Secondary ADP induced platelet aggregation was inhibited in 17 patients after the administration of oral prostaglandin $E_2$ tablets for the induction of labour. Primary ADP induced aggregation was shown to be inhibited by oral prostaglandin $E_2$ when the platelets responded to the lower concentration of 0·5 μg/ml of ADP. This inhibition was not detectable when the higher concentration of 1·0 μg/ml ADP was necessary. There would appear to be a decreased risk of thrombosis initiated by platelet aggregation as a result of using oral $PGE_2$ tablets for inducing labour.

THE role of prostaglandin $E_2$ used for the induction of labour is currently being assessed. Initially the intravenous route was used (Craft *et al.*, 1971; Beazley and Gillespie, 1971), but it has been superseded by the oral administration of prostaglandin $E_2$ solution (Craft, 1972; Kelly *et al.*, 1973; Friedman and Sachtleben, 1974), or more recently by the use of oral prostaglandin $E_2$ tablets (Thiery *et al.*, 1974; Elder and Stone, 1974; Miller *et al.*, 1975).

Factors that can predispose to thrombus formation are increased during late pregnancy, including an increase in ADP induced platelet aggregation (Farbiszewski and Skrzydlewski, 1970).

Intravenous prostaglandin $E_2$ has been shown either to increase the rate of platelet disaggregation (Howie *et al.*, 1973) or to have no effect on the extent of ADP induced platelet aggregation (Karim, 1972). Prostaglandin $E_2$ produced *in vitro* an enhanced effect on secondary ADP induced platelet aggregation (Shio and Ramwell, 1972). As prostaglandin $E_2$ may cause a further increase in platelet aggregation from that already existing in late pregnancy, it is prudent to assess platelet aggregation in women having labour induced by the oral administration of this substance, before it is more widely used.

## PATIENTS AND METHODS

Labour was induced by low amniotomy and a regime beginning with the oral administration of one 0·5 mg. prostaglandin $E_2$ tablet which contained no prostaglandin $E_1$. If contractions had not commenced, this dose of prostaglandin $E_2$ was repeated after one hour, and then by further hourly increments of 0·5 mg. up to a maximum dose of 1·5 mg. per hour. Patients had neither eaten nor smoked during the previous 12 hours, nor had they taken any drugs known to affect platelet aggregation during the previous seven days.

Venous blood was taken from 17 patients before induction of labour and as soon as possible after the onset of established labour, the first sample acting as a control for the second.

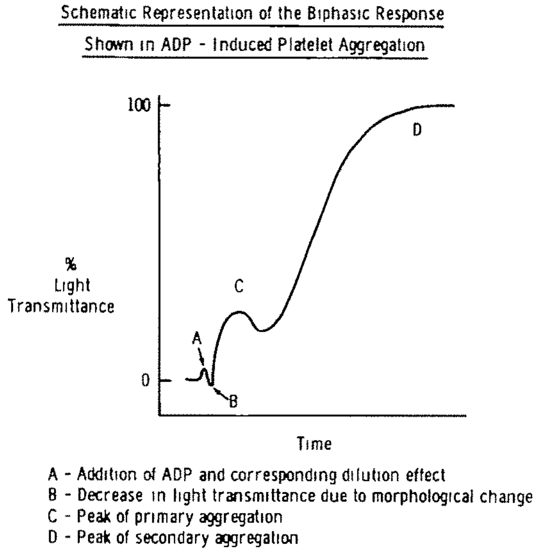
Schematic Representation of the Biphasic Response
Shown in ADP - Induced Platelet Aggregation
100
0
%
Light
Transmittance
Time
A
B
C
D
A - Addition of ADP and corresponding dilution effect
B - Decrease in light transmittance due to morphological change
C - Peak of primary aggregation
D - Peak of secondary aggregation

FIG. 1
Schematic representation of the biphasic response shown in ADP-induced platelet aggregation.

Platelet rich plasma (PRP) and platelet poor plasma was obtained from a 9 : 1 (v/v) mixture of venous blood and 3·1 per cent sodium citrate. The platelet count of the PRP was adjusted to approximately 400 000 per mm.$^3$ by dilution, if necessary, with the subject's own platelet poor plasma.

Platelet aggregation was studied by the method of Born (1962) using an aggregation module (Payton Ltd.) connected to a continuous chart recorder. Aggregation studies were carried out between 60 and 90 minutes after venepuncture. The PRP was kept at room temperature to minimise both the temperature induced shape changes and the variation in subsequent rapid changes in morphological response to aggregating agents.

Aggregation was stimulated by the addition of 0·05 ml. ADP in Owren's buffer, pH 7·35, to 0·45 ml. PRP, the final concentration of ADP in PRP being either 0·5 μg/ml or 1·0 μg/ml. The PRP was allowed to reach 37 °C before the addition of ADP. The dose of ADP used was the lowest required to give a characteristic biphasic aggregation response (Fig. 1).

Identical experimental conditions were maintained for the aggregation studies carried out in duplicate on samples taken both before and during oral prostaglandin $E_2$ induced labour. The same experimental procedure was also applied to three patients who had a spontaneous onset of labour without the administration of oral prostaglandin $E_2$ or intravenous oxytocin. This was to determine the effect on platelet aggregation, of any intrinsic substance that might have been released during normal labour.

The extent of primary and secondary ADP induced aggregation was measured as the per cent increase in light transmittance. The significance of the difference of mean values of platelet aggregation before and after oral prostaglandin $E_2$ administration was tested by Student's "t" test, and by the Mann Whitney "U" test.

## RESULTS

The minimum final ADP concentration required to give the characteristic biphasic aggregation response was 0·5 μg/ml in seven patients, and 1·0 μg/ml in the other ten patients.

The mean extent of secondary ADP induced aggregation as measured by the increase in light transmittance in 17 patients, before and after induction of labour by oral prostaglandin $E_2$ is shown in Table I. At both ADP concentrations secondary aggregation was significantly inhibited after oral prostaglandin $E_2$ administration. An example of this is shown in Figure 2.

The mean extent of primary ADP induced aggregation as measured by the increase in light

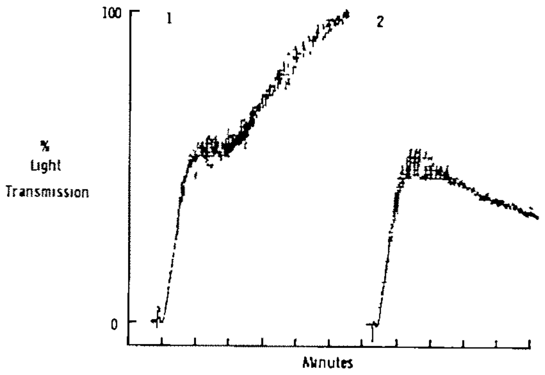

FIG. 2
Inhibition of secondary ADP-induced platelet aggregation by oral prostaglandin $E_2$.
Curve 1 shows secondary aggregation before and curve 2 after the administration of oral prostaglandin $E_2$.

TABLE I

*Mean secondary ADP-induced platelet aggregation as measured by the percentage increase in light transmittance before and after oral prostaglandin E$_2$ administration*

| Final concentration of ADP ($\mu$g/ml) | Number of patients studied | Secondary aggregation before PGE$_2$ (per cent) Mean±SD | Secondary aggregation after PGE$_2$ (per cent) Mean±SD | Significance of difference of means |
|---|---|---|---|---|
| 0·5 | 7 | 86·8±13·8 | 45·6±36·8 | $P<0{\cdot}02$ |
| 1·0 | 10 | 81·8±20·8 | 54·3±27·1 | $P<0{\cdot}05$ |

transmittance in 17 patients, before and after induction of labour by oral prostaglandin E$_2$ is shown in Table II. Those patients who required the lower final concentration of 0·5 $\mu$g/ml of ADP to induce the biphasic response (Fig. 1) showed a significant inhibition of primary aggregation after oral prostaglandin E$_2$ administration. Those who required the higher ADP concentration of 1·0 $\mu$g/ml of ADP did not show any significant inhibition of primary aggregation. There was no significant difference in the amount of prostaglandin E$_2$ ingested up to the time of venepuncture between those patients who required the lower and those who required the higher concentration of ADP stimulus to platelet aggregation ($p>0{\cdot}2$).

The large standard deviations from the mean shown in Tables I and II represent principally patient to patient variation.

Platelets taken from three control patients before and during spontaneous labour, without the use of oxytocic drugs showed no difference in either primary or secondary ADP induced aggregation.

## DISCUSSION

The secondary phase of ADP induced platelet aggregation was inhibited in all 17 patients to such an extent that in some cases only a primary peak of aggregation was obtained (Fig. 2). This suggests that prostaglandin E$_2$ interferes with the release from platelets of substances which are necessary for secondary aggregation, such as ADP and calcium ions. An explanation is that prostaglandin E$_2$ stimulates adenyl cyclase activity which raises cyclic AMP levels and so inhibits this release reaction (Bruno *et al.*, 1974).

Primary ADP induced platelet aggregation is reversible and sensitive to the amount of ADP added. There is a significant inhibition of primary aggregation in those patients requiring the lower ADP stimulus, but not in those requiring the higher ADP stimulus (Table II). The inhibition caused by prostaglandin E$_2$ in the former group may be masked by the higher dose of ADP required to produce biphasic platelet aggregation in the latter group. This implies competition between prostaglandin E$_2$ and ADP for binding sites on the platelet membrane. The rate of absorption and the blood levels of prostaglandin E$_2$ after its ingestion are not yet known. However, there was no significant difference in the mean amount of oral prostaglandin E$_2$ ingested up to the time of venepuncture between patients requiring 0·5 $\mu$g/ml ADP and those requiring 1·0 $\mu$g/ml ADP as the platelet aggregating stimulus.

Secondary aggregation leading to the forma-

TABLE II

*Mean primary ADP-induced aggregation as measured by the percentage increase in light transmittance before and after oral prostaglandin E$_2$ administration*

| Final concentration of ADP ($\mu$g./ml ) | Number of patients studied | Primary aggregation before PGE$_2$ (per cent) Mean±SD | Primary aggregation after PGE$_2$ (per cent) Mean±SD | Significance of difference of means |
|---|---|---|---|---|
| 0·5 | 7 | 26·6± 8·8 | 19·1±11·28 | $P<0{\cdot}05$ |
| 1·0 | 10 | 45·8±19·3 | 42·5±21·3 | NS |

tion of a platelet plug is an important and early factor in thrombus formation. Primary aggregation is a reversible phenomenon which occurs without the release from platelets of endogenous substances such as ADP and calcium ions which are necessary for secondary aggregation. The effects of oral prostaglandin $E_2$ on secondary aggregation seem therefore to be more clinically significant.

Recent *in vitro* experiments have shown that prostaglandin $E_2$ has no effect on the levels of plasma factors V, VII, IX, X, XI and XII (Dubroff *et al.*, 1974).

The results of this study, together with those of Dubroff *et al.* (1974) and Bruno *et al.* (1974), suggest that oral prostaglandin $E_2$ used for the induction of labour may have anti-thrombotic properties and could possibly reduce the incidence of thromboembolic complications of labour and the puerperium.

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# OEDEMA OF THE UMBILICAL CORD AND RESPIRATORY DISTRESS IN THE NEWBORN

BY

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## Summary

Oedema of the umbilical cord, defined as visible oedema in a cord wish a minimal cross sectional area of 1·3 cm$^2$, is found in 10 per cent of deliveries. It is seen more frequently in certain complications of pregnancy such as abrupti placentage, maternal diabetes, macerated intrauterine death and in conditions affecting the infant including prematurity, rhesus isoimmunization, respiratory distress syndrome (RDS) and transient respiratory distress (TRD). There is a higher incidence in infants delivered by Caesarean section. There is no significant association between cord oedema and either fetal distress or neonatal asphyxia nor is there any correlation with maternal hypertension or oedema. The mechanism of production of the oedema is discussed; low oncotic pressure, raised hydrostatic pressure in the placenta and umbilical cord, and an increase in total water in the feto-placental unit are considered. The presence of oedema of the cord may reflect similar changes in the lungs which antenatally predispose an infant whose pathway for production of surfactant is immature to develop RDS and the mature infant to develop TRD. The value of cord oedema as a warning sign is stressed.

IN reviewing the literature on placental pathology one finds oedema of the umbilical cord rarely mentioned (Javert and Barton, 1952; Benirschke and Driscoll, 1967). Isolated case reports of gross oedema (Walz, 1947; Chantler *et al.*, 1969; Howorka and Kapczynski, 1971) or cyst formation (Bergman *et al.*, 1961) can be found, but apart from the case of Chantler *et al.*, (1969) where a patent urachus was present, there has been no attempt to correlate this finding with the state of the infant. Minor degrees of oedema seem to have passed unmentioned, and it is the purpose of the present paper to draw attention to these changes in the cord and their relationship to pregnancy, labour and the state of the infant.

## DEFINITIONS

### *Cord oedema*

The placentae were placed in plastic bags and examined fresh without prior knowledge of the baby's condition. The umbilical cord was measured (Scott and Jordan, 1972) and segments where the vessels were empty were selected. For the purpose of this study two diameters at right angles were measured, multiplied and expressed in cm². Several sites were examined, the maximum value being taken as the cord measurement. Cords with cross sectional areas over $1 \cdot 3$ cm² were considered to be oedematous. Occasionally a cord with marked focal oedema was found. This was included in the oedematous group.

The oedematous cord is easy to identify (Figs. 1 and 2). It has a bloated swollen appearance throughout. Blebs due to local trauma must be differentiated. These frequently show yellowish discolouration and roughening of the amnion, whereas in oedematous cords the surface of the cord is frequently paler and shinier, more like the cord of the extremely premature infant. On section, however, one sees the loose wet jelly-like structure, which is much more translucent than normal Wharton's jelly.

Minor degrees of cord oedema are more difficult to diagnose especially in infants born before 36 weeks. Some of these cords looked oedematous, but were not classified as such if their measurements did not reach the minimal cross sectional requirement ($1 \cdot 3$ cm²).

*Respiratory abnormalities*

Respiratory distress was divided into three groups: (1) respiratory distress syndrome (RDS) was diagnosed if infants had grunting respirations, tachypnoea, rib retraction and radiological signs of a ground glass appearance of the lung fields and an air bronchogram; (2) transient respiratory distress (TRD) was diagnosed if respiratory distress lasted no longer than three days and the chest X-ray was (*a*) normal, or (*b*) showed the pattern of "delayed resorption of pulmonary fluid" (Avery *et al.*, 1966; Kukn *et*

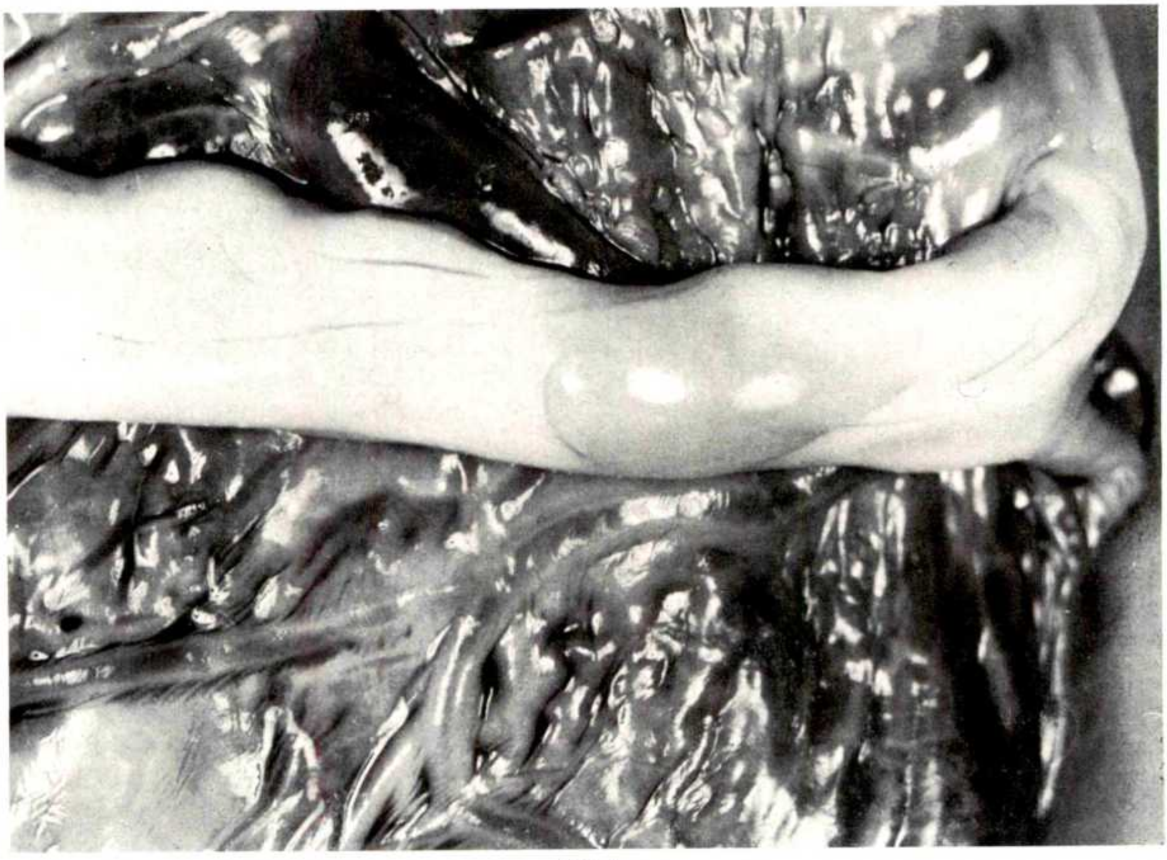

FIG. 1

Oedematous umbilical cord. Note occasional extra "blebs" of oedema.

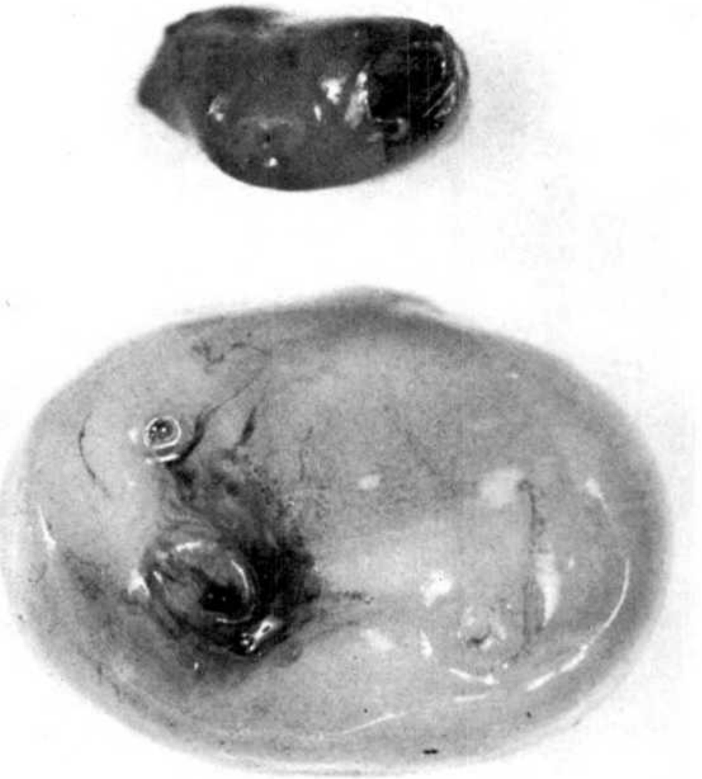

FIG. 2

Cross section of thin wrinkled umbilical cord from small-for-dates baby above and oedematous cord below. This shows the wide range in diameter which can occur.

*al.*, 1969) with hyperaeration, pulmonary vascular prominence, pulmonary oedema and frequently widened interlobar fissures, or (*c*) showed hyperaeration (Sundell *et al.*, 1971) alone or ill-defined infiltrates not suggestive of aspiration pneumonia; (3) aspiration pneumonia was diagnosed if respiratory difficulties were prolonged and the chest X-ray showed definite signs of collapse and consolidation.

Asphyxia was defined as an Apgar score of 6 or less at one minute.

## METHODS AND RESULTS

### *Random series*

A series of 100 placentae were randomly sampled. The incidence of cord oedema (10 per cent) was compared with that found in other conditions, such as rhesus iso-immunization, abruptio placentae, macerated stillbirth and maternal diabetes, in which oedema was known to occur. The results are shown in Table I.

TABLE I

*Incidence of cord oedema in various clinical groups*

| Total No. of patients | Clinical group | Cord oedema No. | Cord oedema Per cent | Comparison with random series |
|---|---|---|---|---|
| 100 | Random series | 10 | 10 | — |
| 50 | Rhesus isoimmunization | 15 | 30 | $P < 0{\cdot}05$ |
| 24 | Abruptio placentae | 15 | 62·5 | $P < 0{\cdot}001$ |
| 25 | Macerated stillbirth | 19 | 76 | $P < 0{\cdot}001$ |
| 17 | Diabetes | 7 | 41·2 | $P < 0{\cdot}001$ |

TABLE II

*Method of delivery in randomly selected series of 100 patients*

| No. of patients | Method of delivery | Umbilical cord |  |  |
|---|---|---|---|---|
|  |  | Normal | Oedematous |  |
|  |  |  | No. | Per cent |
| 10 | Caesarean section | 7 | 3 | 30 |
| 1 | Breech | 1 | 0 | — |
| 26 | Forceps | 23 | 3 | 11·5 |
| 63 | Spontaneous vertex | 59 | 4 | 6·3 |

The random series was then analyzed for causes of cord oedema (Table II), but numbers were too small to draw any definite conclusions, but it seemed that some correlation with Caesarean section might exist.

*Caesarean section*

In order to test this hypothesis the next 50 cases of Caesarean section (Table III) were examined. Here the incidence of cord oedema was found to be 26 per cent, which was significantly higher ($P<0{\cdot}01$) than in the random series (10 per cent). Cord oedema was found in 46·7 per cent of elective as opposed to 17·1 per cent of emergency sections (operations done after the onset of labour), but the numbers were insufficient for statistical analysis.

Asphyxia and TRD seemed to occur more frequently in babies with oedematous cords (Table IV). There were no cases of RDS or aspiration pneumonia. However, the numbers were too small for statistical analysis.

*Admissions to paediatric department*

The case records of 200 consecutive admissions to the Paediatric Department were therefore abstracted, analyzed and correlated with the placental findings. The features associated with RDS, TRD and aspiration pneumonia were analyzed (Table V). Each group was compared with the group showing no respiratory distress using the $\chi^2$ test when numbers were large and

TABLE III

*Incidence of umbilical cord oedema in patients delivered by Caesarean section*

| No. of patients | Clinical group | Umbilical cord |  |  | Comparison with random series |
|---|---|---|---|---|---|
|  |  | Normal | Oedematous |  |  |
|  |  |  | No. | Per cent |  |
| 50 | All Caesarean sections | 37 | 13 | 26 | $P<0{\cdot}01$ |
| 15 | Elective Caesarean section | 8 | 7 | 46·7 | n.s. |
| 35 | Emergency Caesarean section | 29 | 6 | 17·1 | n.s. |
| 100 | Random series | 90 | 10 | 10 | — |

TABLE IV

*Relationship between cord oedema and asphyxia or transient respiratory distress* (TRD)

| No. of patients | Clinical group | Babies with asphyxia |  | Babies with TRD |  |
|---|---|---|---|---|---|
|  |  | No. | Per cent | No. | Per cent |
| 37 | Caesarean section, umbilical cord normal | 15 | 40·5 | 1 | 2·7 |
| 13 | Caesarean section, umbilical cord oedematous | 8 | 61·5 | 3 | 23·1 |
| 90 | Random series, umbilical cord normal | 13 | 14·4 | 1 | 1·1 |
| 10 | Random series, umbilical cord oedematous | 3 | 30 | 1 | 10 |

There were no cases of RDS or aspiration pneumonia

TABLE V

*Incidence of respiratory distress in relation to cord oedema in 200 infants*

| No. of patients | Clinical group | Umbilical cord |  | Comparison with random series |
|---|---|---|---|---|
|  |  | Normal | Oedematous |  |
| 146 | No respiratory distress | 126 | 20 | — |
| 14 | RDS | 5 | 9 | $P<0\cdot001$ |
| 34 | TRD | 23 | 11 | $P<0\cdot05$ |
| 6 | Aspiration pneumonia | 6 | 0 | — |
| 200 | Total | 160 | 40 | — |

Fisher's exact test for small numbers. Of 54 cases with respiratory distress, 20 had cord oedema. There was a significant correlation between RDS and TRD and the presence of cord oedema. None of the six babies with aspiration pneumonia had cord oedema.

As previously stated, the significance of cord oedema was more difficult to assess in infants born before 36 weeks. Nevertheless of the 25 infants, less than 36 weeks gestation in the above series of 200 paediatric admissions, 11 (44 per cent) had cord oedema and eight of them had respiratory distress. Only three of the remaining 14 preterm infants with no cord oedema had respiratory distress. This difference was significant at the 5 per cent level.

Maternal hypertension, oedema and fetal distress, were similarly reviewed but no correlation with cord oedema was found. There were no cases of funisitis. Neonatal asphyxia was common, but no statistically significant association between it and cord oedema was found.

### *Sex ratio*

In the random series the male/female ratio was 52 : 48 and the same ratio for the ten infants in this group with cord oedema was 8 : 2; the difference is not statistically significant ($P<0\cdot1$). The male/female ratio for infants who had an oedematous umbilical cord on admission to paediatric care was 2·3 : 1, but this may be explained by the high male/female ratio caused by RDS or asphyxia.

## DISCUSSION

Several factors might be responsible for oedema of the umbilical cord: foremost among these is a low plasma oncotic pressure due to reduced plasma proteins or actual leakage of protein from the vessels with build up of oncotic pressure in the interstitial space; alternatively, raised hydrostatic pressure within the placental and umbilical cord vessels may lead to transfer of fluid into the cord substance; finally, there may be an increase in the total water content of the feto-placental unit. These various factors will be discussed in relation to the findings in the present survey.

These show that oedema of the cord occurred more often in preterm than in term infants, more in infants delivered abdominally (30 per cent) than vaginally (6·3 per cent), and probably more often in infants delivered by elective Caesarean section than in those delivered by emergency Caesarean section (Table III). It would appear that dynamic changes in the placenta, umbilical cord and fetus occur during vaginal delivery and that all these changes are to the infant's benefit. The serum proteins (Davidsen, 1971; Bland, 1972) and IgG (Jones and Payne, 1967) in the cord blood of infants delivered by Caesarean section are lower than those born by vaginal delivery and in elective as distinct from emergency section, serum trypsin inhibitor levels are lower (Kotas *et al.*, 1972). Cassady (1971) found significantly increased total body water in infants delivered abdominally rather than vaginally.

Asphyxia caused by umbilical cord occlusion in lambs results in leakage of fluid from vascular spaces (Adamson *et al.*, 1970) and it would be reasonable to assume a similar effect in the human fetus. Some focal oedema is usually noted in relation to true knots of the cord; such cases were, however, excluded from the present survey. Brinkman (1970) also showed changes in the vascular resistance in the umbilical sinus and placenta vessels in relation to asphyxia. In this series we were unable to show any statistically significant association between asphyxia and cord oedema, nor was any correlation noted between fetal distress, maternal hypertension or oedema and cord oedema. Positive associations existed, however, for abruptio placentae, maternal diabetes, prematurity, rhesus isoimmunization, RDS and TRD.

In abruptio placentae the oedema occurred most commonly when the fetus was stillborn and the retroplacental haematoma large. In such cases there is compression of the villous capillary bed which might lead to a possible rise in hydrostatic pressure within the umbilical vessels and eventual oedema. On the other hand Bland (1972) found low serum proteins in the umbilical cord blood of infants born following third trimester haemorrhage but the number of cases of abruptio placentae was not stated so it is possible that a dual pathology was involved.

The cord oedema in infants of diabetic mothers is more difficult to explain. The level of serum proteins in these infants is not significantly different from that of others of similar gestational age (Davidsen, 1971). It may be that there is some alteration in ground substance which allows fluid to pass out of vessels and holds it in the interstitial space (Muir, 1964).

The cord oedema in preterm infants and severe rhesus iso-immunization can be explained by the low oncotic pressure which has already been reported (Baum *et al.*, 1971; Baum and Harris, 1972). It has been suggested that in hydrops fetalis oedema might be related to cardiac failure but recent studies have not shown any increase in blood volume (Maclaurin *et al.*, 1974). Certainly, in preterm infants the low oncotic pressure due to reduced production of serum proteins is aggravated by the increased capillary permeability (Krasilnikoff, 1969).

In the present paper the association between cord oedema, RDS and TRD (Table V) is interesting. Since the finding by Avery and Mead (1959) of reduced surfactant in infants dying of hyaline membrane disease much attention has been paid to this factor (Reynolds *et al.*, 1968; Boughton *et al.*, 1970; Kotas, 1972). Yet the aetiology of RDS still remains unknown. It is generally held that immature infants have sufficient surfactant activity available at 28 to 30 weeks gestation (Reynolds *et al.*, 1968; Boughton *et al.*, 1970; Gandy *et al.*, 1970) to survive without developing RDS but demand outstrips supply. Asphyxia (Reynolds *et al.*, 1968; Hey and Hull, 1971) has been considered a precipitating factor in predisposing an infant to RDS, perhaps by interfering with surfactant production, but it cannot be held responsible for those cases without evidence of fetal distress or birth asphyxia. The finding of lower serum proteins (Markarian *et al.*, 1966; Hardie *et al.*, 1968; Bland, 1972) and low alpha-1-antitrypsin (Kotas, *et al.* 1972; El-Bardeesy *et al.*, 1972; Evans *et al.*, 1972) in the cord blood of infants that later develop RDS suggests that these factors may play a role and the association between oedema of the cord and RDS or TRD support this concept.

TRD has been given a variety of names: type II respiratory distress syndrome (Prod'hom *et al.*, 1965), neonatal disseminated atelectasis (Heese *et al.*, 1966), transient tachypnoea of the newborn (Avery *et al.*, 1966), transient respiratory distress of the newborn (Swischuk, 1970), wet lung disease (Wesenberg *et al.*, 1971), respiratory distress type II (Sundell *et al.*, 1971) and benign respiratory distress (Taylor *et al.*, 1971). From a review of the literature and our own experience the following factors are seen to be common to TRD and oedema of the cord: both occur in mature infants; both show a higher incidence of Caesarean section and a slightly higher incidence of asphyxia; both affect more males than females. Serum proteins are low in TRD (Wesenberg *et al.*, 1971; Steele and Copeland, 1972). It is also interesting to note that Steele and Copeland (1972) found that the radiological appearance of "delayed resorption" of pulmonary alveolar fluid (Avery *et al.*, 1966; Kukn *et al.*, 1969) occurred in 5·7 per cent of newborn and that only 16·6 per cent of these had symptoms although all had low serum proteins.

The aetiology of TRD is probably multifactorial and as the prognosis is excellent and mortality negligible it is difficult to investigate. Sundell *et al.* (1971) thought that their cases with prominent hyperaeration were due to lower airway obstruction and to failure to clear the lungs of accumulated mucus and debris. In the cases with radiological features of "delayed resorption" and low serum proteins there may be a transcapillary shift of proteins and fluid into the interstitial or alveolar spaces resulting in pulmonary oedema (Taylor *et al.*, 1971) rather than primarily "delayed resorption". It is possible that oedema of the umbilical cord reflects changes already existing in the lungs. This being so, one might postulate that a "wet lung" predisposes the infant with an immature pathway of surfactant production to develop RDS whereas the more mature baby may be more liable to develop TRD. Further investigations along these lines are proceeding. Meanwhile we stress the significance of oedema of the cord and would suggest that cord oedema should put those caring for the newborn on the alert for respiratory distress.

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# CHANGES IN PLASMA NON ESTERIFIED FATTY ACIDS AND SERUM GLYCEROL IN PREGNANCY

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## Summary

Changes in plasma nonesterified fatty acids (NEFA) and serum glycerol in pregnancy were examined in a semi-serial study of 85 women. A preliminary study showed that it was almost impossible to achieve standardized conditions for sampling, and the compromise of taking a single venous sample without stasis, after 30 minutes rest and about 12 hours fasting, was adopted. Although there were large individual variations the patterns of change were, in general, the same for NEFA and glycerol although the two were not closely correlated. There was no convincing change before 30 weeks of pregnancy but both increased in the last ten weeks, fell sharply in the first week *post partum*, and then rose again to late pregnancy levels by 6 weeks *post partum* before falling to non-pregnant levels by between 3 and 6 months *post partum*. Those patterns of change are in broad accord with changes of fat storage and lipolysis associated with the reproductive cycle. Changes in the patterns of NEFA were trivial; the only significant alteration was a small rise in the proportion of C16:0 (palmitic) in late pregnancy.

THE large and widespread upheaval of metabolism in normal pregnancy affects every nutrient; some appear to be influenced by a primary change in homeostatic control, others may change secondarily and only because of their interdependence.

The circulating level of non-esterified (or free) fatty acids (NEFA) is affected by, and in turn affects, many aspects of nutrient metabolism so that prediction of their behaviour in pregnancy is difficult. The pattern of change in NEFA concentration during pregnancy is not well documented and most published data refer to late pregnancy when there is general agreement that levels are raised (Burt, 1960; Bleicher *et al.*, 1964; Gualandi, 1964; Nelson, 1965; Picard *et al.*, 1967; Fioretti *et al.*, 1970; Fairweather, 1971; Potnis and Purandare, 1972). Almost all the published information was obtained by colorimetric or titrimetric methods of varying specificity (McDonald-Gibson and Young, 1974) and it is at least possible that the well recognized rise in other plasma lipids, particularly phospholipid (Quinto *et al.*, 1967; Becker *et al.*, 1971; Van Duyne and Havel, 1960; Dannenburg and Burt, 1965) and organic acids (Dannenburg and Burt, 1965; Svanborg and Vikrot, 1965) may have contributed to an apparent rise in NEFA.

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In this study we have used a specific gas chromatographic method (McDonald-Gibson and Young, 1974) to measure NEFA in a semi-serial study of healthy pregnant women. Glycerol has also been measured as a "marker" of lipolysis (Steinberg, 1963); a rise in NEFA without a concomitant rise in glycerol could suggest *de novo* synthesis of NEFA rather than lipolysis or a decrease in fatty acid utilization. Indeed glycerol, which unlike NEFA cannot be utilized by the adipose tissue cell for rebuilding neutral fat (Björntorp, 1967; Shapiro *et al.*, 1957), may be a better indicator of lipolysis than NEFA (Vaughan and Steinberg, 1963).

## METHODS

### *The standardization of blood samples*

A measurement of NEFA or glycerol level in the plasma is valid only for the circumstances at the time of sampling. There is no physiological situation analogous to the basal or fasting state for plasma glucose where homeostatic forces ensure an almost constant level; NEFA and glycerol are extremely "labile", rising continuously during fasting (Gordon and Cherkes, 1956; Pinter and Pattee, 1969) and highly sensitive to numerous other influences including the effects of exertion (Greaves *et al.*, 1972; Carlson *et al.*, 1963); emotional stress (Bogdonoff *et al.*, 1959), the temperament of the subject and whether or not she smokes cigarettes, venous stasis and even the local effect of an indwelling needle.

Many of these effects were demonstrated in a preliminary investigation to find the best "standard" conditions under which to sample our pregnant subjects.

Large and unpredictable changes of as much as half the mean value could occur a few minutes apart, even when the "stress" of repeated venepuncture was avoided by the use of an indwelling canula. Similar degrees of variation occurred when the same subjects were sampled under similar fasting conditions at daily or weekly intervals.

The possibility that fatty acid levels might be more labile in the fasting state than in the fed state was also tested by sampling women at a fixed time after a standard glucose load but there was no evidence that this led to a more stable state.

The conclusion was that no truly standardized conditions were possible, but the most convenient compromise seemed to be that the subjects should be fasting from 22.00 hours on the night before, come to the Unit at about 09.00, sit quietly for 30 minutes and then have a single sample taken by rapid venepuncture with minimum stasis.

### *Laboratory procedures*

Ten ml. of blood were taken by plastic syringe, seven ml. dispensed immediately into an ice cold heparinized plastic tube for NEFA determination, and three ml. into a cold glass tube for glycerol determination.

Plasma for NEFA determination was immediately prepared by centrifugation at 4 °C. and the NEFA extracted without delay. The plasmas were never stored since changes can occur even at −20 °C. (Howarth *et al.*, 1966; Zieve and Vogel, 1961; Munker, 1959). Serum for glycerol determination was obtained by allowing the blood to clot at 4 °C.; it was then stored at −20 °C. for up to one week; glycerol was found to remain stable at this temperature for at least a week.

*Determination of NEFA*: Plasma NEFA were determined as described by McDonald-Gibson and Young (1974). In brief, NEFA were extracted from plasma by the method of Dole and Meinertz, (1960), separated from other lipids by thin layer chromatography (Fosbrooke and Tamir, 1968) and methylated by the method of Morrison and Smith (1964). Thereafter determination was by a quantitative gas liquid chromatographic method using a pentadecanoic acid internal standard. The instrument was a Pye 104 gas chromatograph equipped with a flame ionization detector and an Auto Solids Injection System. Reproducibility of the method was high: the coefficient of variation of repeated measurements being between 3 and 4 per cent.

*Glycerol* was estimated by an enzymic method (Eggstein and Kreutz, 1966) using the kit supplied by Boehringer Mannheim GMBH. The assay was highly reproducible, the coefficient of variation of repeat estimations being about 1 per cent at normal serum levels and less than 0·3 per cent at high levels.

LIBRARY

*Subjects*

Fifteen pregnant women in good health formed a longitudinal series in which samples were taken at about 13, 20, 30 and 38 weeks of gestation, and at 3 to 7 days, 6, 12 and 24 weeks after delivery; one of the fifteen also provided a pre-pregnancy sample. Seventy other healthy pregnant and non-pregnant women provided samples, many of them on more than one occasion.

## RESULTS

Table I shows the means and standard deviations for the two groups of patients. Although the mean values do not show an exact correspondence of patterns they have some important points in common. For both NEFA and gylcerol, there was an increase from 30 to 38 weeks of pregnancy, a fall from late pregnancy to 3 to 7 days *post partum* followed by a rise to late pregnancy levels at 6 weeks post partum and a subsequent fall. The rather large between-patient variability at all time points should also be noted.

Since, as mentioned previously, a number of patients in the non-longitudinal group provided more than one sample, a better idea of changes between the time points may be obtained by utilizing fully the longitudinal element of the study. Table II shows mean changes in NEFA and glycerol between consecutive time points. The features described already for Table I are

TABLE I

*NEFA and glycerol values for selected time points. Number of patients at each point in brackets*

| | NEFA | | Glycerol | |
|---|---|---|---|---|
| Time: | Longitudinal Mean ± SD | Other Mean ± SD | Longitudinal Mean ± SD | Other Mean ± SD |
| Non Pregnant | 480 (1) | 428±119 (27) | 48 | 50±17 |
| Pregnant | | | | |
| 13 weeks | 413±84 (14) | 422±230 (8) | 44±14 | 42±23 |
| 20 weeks | 370±147 (15) | 426±80 (9) | 38±9 | 45±13 |
| 30 weeks | 336±87 (15) | 450±78 (19) | 39±14 | 41±14 |
| 38 weeks | 422±89 (15) | 496±131 (31) | 50±18 | 54±25 |
| Post partum | | | | |
| 3–7 days | 341±143 (14) | 333±123 (16) | 43±16 | 41±14 |
| 6 weeks | 468±122 (15) | 534±159 (29) | 54±17 | 56±23 |
| 12 weeks | 383±96 (14) | 432±162 (26) | 49±14 | 43±14 |
| 24 weeks | 328±107 (13) | 359±107 (21) | 42±12 | 46±15 |

TABLE II

*Changes in NEFA and glycerol levels between consecutive time points. (* = significant at 5 per cent; ** = significant at 1 per cent; number of patients at each point shown in brackets)*

| Change: from | to | NEFA Mean ± SD | Glycerol Mean ± SD |
|---|---|---|---|
| 13 weeks | 20 weeks | −19±183 (18) | −1·0±17 2 |
| 20 weeks | 30 weeks | −8±131 (23) | 6 0±13·3 |
| 30 weeks | 38 weeks | 63±105 (28)** | 13·3±16·5** |
| 38 weeks | 3–7 days P.P. | −103±147 (26)** | −10·2±25·3* |
| 3–7 days P.P. | 6 weeks post partum | 145±203 (29)** | 12·5±27 0* |
| 6 weeks post partum | 12 weeks post partum | −81±166 (36)** | −7·8±23·7 |
| 12 weeks post partum | 24 weeks post partum | −69±160 (33)* | −2·4±14·1 |

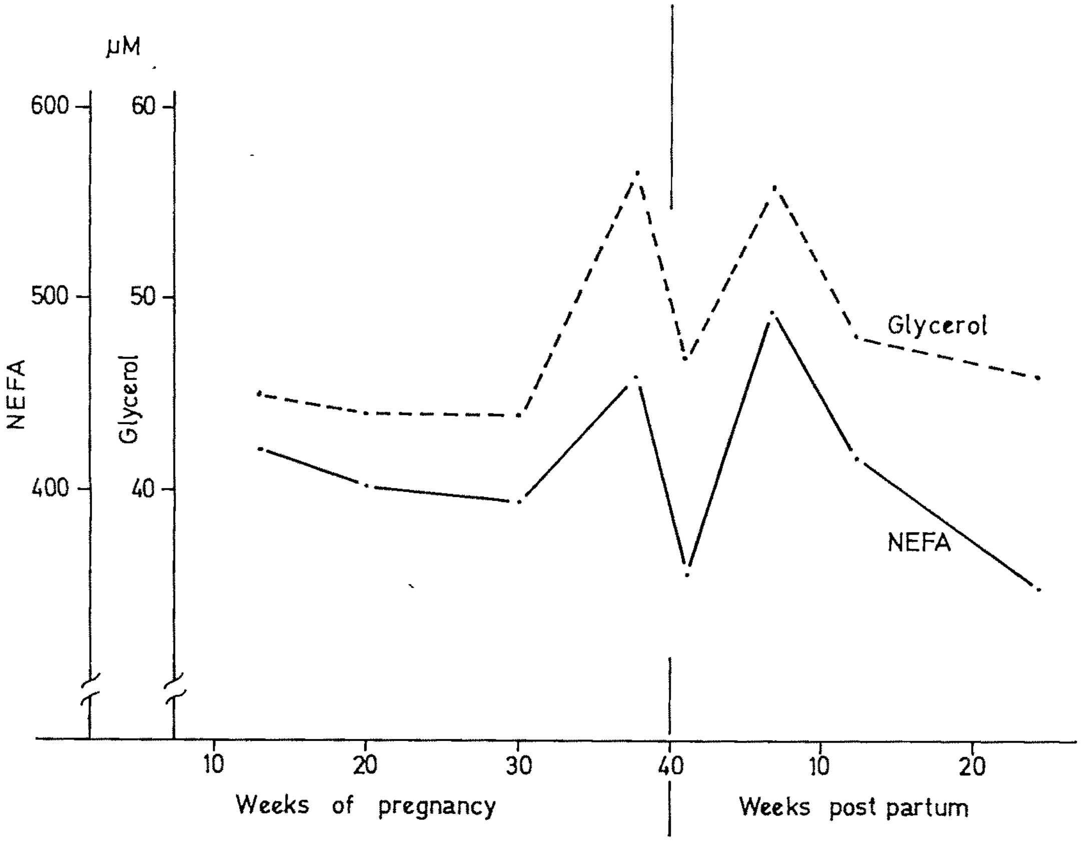

FIG. 1
Idealized Pattern of NEFA and Glycerol Changes in Pregnancy derived from Table II.

shown to attain statistical significance for NEFA from 30 weeks of pregnancy onwards, but for glycerol significant changes are observed only for differences between 30 weeks of pregnancy and 6 weeks *post partum*. Using changes shown in Table II we may construct characteristic curves for NEFA and glycerol as shown in Fig. 1. The majority of patients showed a rise from 30 to 38 weeks of pregnancy, a fall from late pregnancy to 3 to 7 days *post partum* and a rise to 6 weeks—there can be little doubt about the reality of these changes. Since there were no pairs of samples for early pregnancy and the non-pregnant state, apart from those at 24 weeks *post partum* when a stable non-pregnant state may not have been reached, there is no way of knowing whether the value at 13 weeks of pregnancy differs from that immediately before pregnancy.

The rough overall concordance between NEFA and glycerol conceals many discrepancies and the correlation between the two, within individual subjects, was only 0·54.

As might have been expected from the preliminary study which demonstrated huge variations occurring from time to time in the same individual, the overall trends conceal many individual patterns of change; indeed only three of the fifteen individuals studied serially behaved in the average fashion. Because of this biological "noise" the examination of correlations between NEFA and glycerol and such clinical variables as the subject's weight and weight gain has proved totally unprofitable; very large numbers would be needed to show a trend, if one exists.

In the non-pregnant and postpartum groups differences were looked for between those who were, or were not taking oral contraceptives, and between those who were or were not lactating; no significant differences were found.

The relatively high early pregnancy values, particularly obvious in the longitudinal series when compared to mid pregnancy (Table I) were a reflection of a few very high individual values. The possibility was examined that these differences may have been due to negative energy balance in some women due to nausea and vomiting; but the differences in pattern between women who reported vomiting in early pregnancy and those who did not was unconvincing.

Table III shows the pattern of change in the individual fatty acids which made up the total, expressed as percentages of that total. The proportion of C16:0 (palmitic) shows an increase in late pregnancy and that of C18:1 (oleic) a slight decrease; only the difference for C16:0 is significant.

## DISCUSSION

Overall, the changes described were so similar for NEFA and glycerol that it can be reasonably assumed that they resulted from variations of lipolysis of adipose tissue triglycerides. Apart from Konttinen and Pyorälä (1964) who found no change and Dražančić and Stavlenic (1971) who found a very small and statistically doubtful change, previous studies have been unanimous in showing that NEFA levels in late pregnancy are considerably above those in early pregnancy or in the non-pregnant woman (Burt, 1960; Bleicher *et al.*, 1964; Gualandi, 1964; Nelson, 1965; Picard *et al.*, 1968; Fioretti *et al.*, 1970; Fairweather, 1971; Potnis and Purandare, 1972). Beyond that simple fact, unanimity ceases. Mean levels in late pregnancy cover a range from under 600 $\mu$M (Dražančić and Stavlenic, 1971) to 1255 $\mu$M( Burt, 1960). Picard *et al.*, (1968) and Fairweather (1971) showed a rise in early pregnancy compared to the non-pregnant; Potnis and Purandare (1972) showed a fall which, curiously, they attributed to vomiting by two-thirds of their subjects.

All previously published studies have used titration or colorimetric methods of varying

TABLE III

*Percentage composition of total non esterified fatty acids (NEFA)*

| Non esterified fatty acid: | | Not pregnant | Pregnant (weeks) | | | | Post-partum | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 13 | 20 | 30 | 38 | 3–7 days | 6 weeks | 12 weeks | 24 weeks |
| N | | 28 | 22 | 24 | 34 | 46 | 30 | 44 | 40 | 34 |
| C14.0 | Mean | 1·97 | 2·13 | 2 33 | 2·29 | 2·37 | 2·28 | 2·20 | 2·18 | 2 18 |
| | SD | 0·42 | 0·46 | 0·54 | 0·51 | 0·47 | 0·45 | 0·60 | 0·49 | 0·57 |
| C16:0 | Mean | 26·02 | 25·54 | 26·30 | 27·25 | 27·53 | 27·26 | 25 99 | 25·80 | 26·58 |
| | SD | 1·19 | 1·26 | 1·84 | 2·17 | 1·75 | 1·80 | 1·75 | 1 47 | 2·49 |
| C16:1 | Mean | 5·46 | 5·27 | 5 21 | 5·32 | 5·40 | 6·12 | 5·99 | 5·82 | 5·20 |
| | SD | 0·94 | 0·79 | 1·42 | 0 93 | 0·93 | 1·13 | 1·15 | 1·27 | 1·10 |
| C17:0 | Mean | 0·00 | 0 00 | 0·00 | 0·02 | 0·02 | 0·00 | 0·00 | 0 00 | 0·00 |
| | SD | — | — | — | 0·07 | 0·08 | — | — | — | — |
| C18:0 | Mean | 13·38 | 14·18 | 13·91 | 13·66 | 13·29 | 11·98 | 12·90 | 12·93 | 14·06 |
| | SD | 1·77 | 2·03 | 2·46 | 2·02 | 1·90 | 2·16 | 2·75 | 2·35 | 1·96 |
| C18:1 | Mean | 42·01 | 41·57 | 40·86 | 40·48 | 40·59 | 42·78 | 42·78 | 42·44 | 41·90 |
| | SD | 1·85 | 2·52 | 3·61 | 2·71 | 2·62 | 2·71 | 2·48 | 2·43 | 2·77 |
| C18:2 | Mean | 8·76 | 8 47 | 8·69 | 8·24 | 8·09 | 7·29 | 7·80 | 8·37 | 8·13 |
| | SD | 1·45 | 1·60 | 2 40 | 1·80 | 1·70 | 1·13 | 1·39 | 1·67 | 1·98 |
| C18:3 | Mean | 1·71 | 1·87 | 1·91 | 1·81 | 1·74 | 1·68 | 1·72 | 1·74 | 1·43 |
| | SD | 0·38 | 0·31 | 0·27 | 0·35 | 0·48 | 0·27 | 0·38 | 0·29 | 0·58 |
| C20:0 | Mean | 0·01 | 0·01 | 0·00 | 0·01 | 0·01 | 0·00 | 0·01 | 0 00 | 0·00 |
| | | – | – | – | – | – | – | – | – | – |
| C20:4 | Mean | 0·57 | 0·77 | 0·56 | 0·67 | 0·71 | 0·40 | 0·43 | 0 50 | 0·41 |
| | SD | 0·21 | 0·27 | 0·29 | 0·41 | 0 69 | 0·58 | 0·55 | 0·43 | 0·48 |
| C22:0 | Mean | 0·01 | 0·00 | 0·00 | 0 01 | 0·04 | 0·02 | 0·03 | 0·01 | 0·00 |
| | | – | – | – | – | – | – | – | – | – |
| Total | | 99·90 | 99·81 | 99·77 | 99·76 | 99·79 | 99·81 | 99·85 | 99·79 | 99·89 |

Very small unidentified peaks have been omitted, thus the totals do not add up to exactly 100 per cent.

specificity, mostly variations of the method of Dole (1956) and Duncombe (1963), and all except Fairweather (1971) had been cross sectional. It was the hope that the present study, by using a specific gas chromatographic method for NEFA and by the use of at least partially serial sampling, would give a more definitive picture. That hope has not been realized and our experience in attempting to standardize the conditions for sampling, suggests that its achievement may not be possible.

One interpretation of the fall in NEFA seen during early and mid-pregnancy in the serial subjects, and their generally lower levels compared to the non-serial group could be that they had lost their apprehension of the venepuncture after the first one or two samples. In that sense serial sampling except in experienced subjects, may give misleading results, and it is of interest that Fairweather (1971), who reported the only other serial study, also found the first early pregnancy sample to be considerably above the mid-pregnancy levels.

The general pattern of change described here makes physiological sense, the low and perhaps even below-normal levels in mid pregnancy are consistent with the storage of neutral fat in adipose tissue which occurs at that time; and the rising levels in late pregnancy reflect the cessation of storage and the beginning of lipolysis which typifies the last trimester (Hytten and Leitch, 1971). Such a pattern accords closely with that shown for the rat by Knopp *et al.*, (1973): in the first half of pregnancy there is twice the usual conversion of glucose to storage triglyceride and a dimished release of NEFA; in the second half of pregnancy, as insulin effectiveness deteriorates, the formation of fatty acids from glucose falls and maternal fat stores are increasingly mobilized.

The dramatic peak at six weeks *post partum* also occurs at a time when the fat stored in pregnancy is being broken down. Why the levels in the first week *post partum* should be low is difficult to explain; the early puerperium is typically a time of enormous metabolic readjustment, the details of which are almost unknown.

There were only minor changes in the patterns of individual fatty acids during pregnancy. Previous studies (Hansen *et al.*, 1964; Quinto *et al.*, 1967) have shown a relative rise in the proportion of saturated acids, palmitic and stearic, and a relative fall in unsaturated acids, oleic, linoleic and arachidonic. Our study also shows a very small rise in palmitic acid (C16:0) and a very small, not statistically significant fall in oleic acid (C18:1). There is no hint of a fall in other unsaturated acids, indeed arachidonic acid (C20:4) shows, if anything, a slight increase. The biological significance of these small changes, if they are real, is not known.

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# THE RELATION BETWEEN MATERNAL AND CORD SERUM IRON LEVELS AND ITS EFFECT ON FETAL GROWTH IN IRON DEFICIENT MOTHERS WITHOUT MALARIAL INFECTION

BY

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## Summary

In this relatively iron deficient population both multiparous and primiparous pregnant women had similar serum iron levels. The mothers' levels had a linear correlation with the cord serum levels, but the lowest mean cord levels were above 100 μg./dl. Maternal and cord blood haemoglobin levels, birthweights and placental weights showed no constant relation to the serum iron levels, and even in anaemic mothers the fetus was relatively non-anaemic with a haemoglobin level around 13 g./dl. In non-anaemic mothers the cord blood haemoglobin rose rapidly and progressively.

FETAL growth depends to a certain extent on the availability of iron from the mother. Although the fetus is an efficient parasite, in relatively anaemic mothers with low haemoglobin levels and low serum iron levels, it is not clear if the fetus takes the optimum amounts of iron or if it takes amounts proportional to the levels available in the mother. Conflicting results have been reported on the effects of iron deficiency on the newborn. Sturgeon (1959) showed that the cord blood haemoglobin levels were similar in those from anaemic and non-anaemic mothers. On the other hand Sisson and Lund (1957) observed a significant decrease in haematocrit values, haemoglobin values, and plasma iron levels in children delivered by iron deficient mothers. In a WHO (1968) study it was shown that even if a saturation of transferrin below 15 per cent was used as a criterion of iron deficiency the incidence in pregnancy in different parts of the world was over 40 per cent. In this area 50 to 70 per cent of expectant mothers have a very low serum iron and a transferrin saturation of 15 per cent or less throughout pregnancy (Nhonoli, 1974).

It was therefore decided to find out the relation between the maternal and fetal serum iron levels in those patients proved to be free of malarial infection.

## MATERIALS AND METHODS

The subjects were 580 African women who had singleton livebirths of between 38 and 40 weeks gestation in Dar es Salaam during May and June 1973. Maternal venous blood was taken during the first stage of labour, and cord blood was collected. The placenta was wiped clean, an incision made about 2 cm. deep, and blood was collected from the base of the incision; thick and thin films were prepared. The cord was cut to within 2 cm. of its insertion, all loose membranes removed, clots wiped out and the placenta weighed to within 25 g. The infant was similarly weighed. Thin and thick blood smears were prepared from the heel prick of the infant immediately after birth. Similar smears were prepared from the cord blood and maternal blood. Maternal and cord blood haemoglobin was estimated by the cyanmethemoglobin method. Serum iron was estimated on the maternal and cord blood by the method of Ramsey (1957) using the newer chromogen 0·2 per cent tripyridyl and read on a Unicam SP 600 spectrophotometer at 520 nm. The blood slides were examined for malarial parasites by a single experienced technician; the technique used in making slides and staining with Giemsa's stain was that of Shute and Maryon (1966).

Only serum iron was estimated; no total iron binding capacity (TIBC) was done because the current concept is that serum iron levels are more reliable than TIBC levels in pregnancy (Rybo, 1973).

## RESULTS

Of the 580 single births investigated at the completion of the study complete results were available for only 475. Of the 475 with complete records only 432 were found to be free from malaria infection in all four specimens of blood, namely, from the mother, the placenta, the umbilical cord, and the infant. As malarial haemolysis affects serum iron levels as well as fetal and placental weights, only those 432 deliveries free of malarial infection were included in computing the results.

### *Relation of maternal serum iron to placental weight and birthweight*

This was investigated in 330 multiparae. Of 175 with male infants, 88 had serum iron levels of 80 μg./dl. or less and mean birthweight was 3235±348 (SD) and placental weight 428±69 g.; 87 had serum iron levels of 81 to 180 μg./dl., and the mean birthweight was 3251±416 g., placental weight 440±62 g.

In 155 with female infants, 69 had serum iron levels of 80 μg./dl. or less and the mean birthweight was 3021±434 g., placental weight 422±98 g.; 86 had serum iron levels of 81 to 180 μg./dl., and the mean birthweight was 3102±393 g., placental weight was 409±84 g.

These results show no obvious relation between the level of maternal serum iron and either the birthweight or the placental weight.

### *The relation between maternal and cord serum iron levels*

Table I and Figure 1 show this relationship in 340 multiparae. It is a linear correlation, the grouped data coefficient of correlation (r) is +0·5868, the estimating equation being $c = 78{\cdot}080 + 0{\cdot}8865X$ and the standard error of the estimate is 45·32. Thus the cord serum iron rises directly as the maternal serum iron rises. In these 340 patients the mean maternal serum iron was 83±42 μg./dl., and the mean cord serum iron was 158±60 μg./dl. Thus the fetus is an efficient parasite reaching high mean levels despite the fact that the mean maternal levels were subnormal.

### *Composite inter-relationships between maternal serum iron, cord serum iron, birthweight, maternal haemoglobin, cord blood haemoglobin and placental weight*

Table II gives these inter-relationships in a detailed mathematical form for all 432 multiparae and primiparae combined. Many of the mothers appear to be iron deficient, 72·5 per cent having serum iron levels below 100 μg./dl., 50·5 per cent with levels below 80 μg./dl., and 15 per cent with less than 40 μg./dl. Birthweights and placental weights are clearly unrelated to the

TABLE I

*Relation of maternal serum iron to cord serum iron levels in multiparous mothers*

| Number (Total=340) | 46 | 69 | 55 | 88 | 29 | 24 | 20 | 7 | 2 |
|---|---|---|---|---|---|---|---|---|---|
| Range of maternal serum iron (μg./dl.) | 21–40 | 41–60 | 61–80 | 81–100 | 101–120 | 121–140 | 141–160 | 161–180 | 181–200 |
| Mean cord blood serum iron (μg.dl.) | 103 | 126 | 134 | 163 | 161 | 182 | 217 | 247 | 270 |

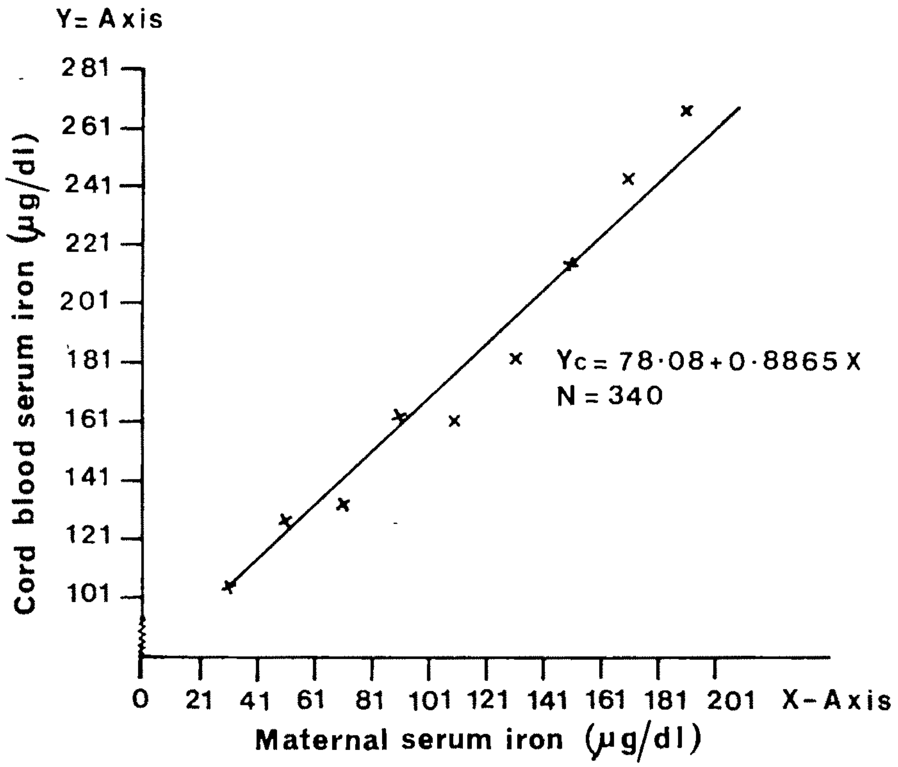

FIG. 1

Relationship of maternal serum iron to cord blood serum iron levels in all singleton full term births (38 to 40 weeks) in mothers without evidence of malarial infection.

TABLE II

*Birthweight, placental weight, maternal and cord haemoglobin concentration and cord serum iron, related to maternal serum iron concentration*

| Range of maternal serum iron (µg./dl.) | Number | Cord blood serum iron (µg./dl.) Mean±SD | Birthweight | | No. of mothers with haemoglobin below 9·9 g./dl. (per cent) | No. of cord blood haemoglobin levels below 13 4 g./dl. (per cent) | Placental weight (g.) Mean±SD |
|---|---|---|---|---|---|---|---|
| | | | Mean ±SD (g.) | Per cent below 2·5 kg. | | | |
| 21–40 | 64 | 125±64 | 3212±440 | 4·68 | 39·0 | 57·8 | 433±67 |
| 41–60 | 84 | 134±56 | 3140±391 | 5·95 | 34·5 | 59·5 | 415±74 |
| 61–80 | 70 | 141±58 | 3100±458 | 10·00 | 34·3 | 41·4 | 417±84 |
| 81–100 | 95 | 163±57 | 3118±431 | 6·31 | 33·6 | 45·2 | 414±68 |
| 101–120 | 34 | 170±50 | 3206±448 | 2·94 | 44·1 | 52·9 | 418±68 |
| 121–140 | 28 | 179±46 | 3180±385 | 3·57 | 25·0 | 57·1 | 445±86 |
| 141–160 | 27 | 227±60 | 3107±346 | 7·40 | 22·2 | 55·5 | 421±61 |
| 161–180 | 30 | 237±65 | 3044±408 | 13·33 | 33·3 | 46·6 | 411±59 |
| Total | 432 | | | | | | |

TABLE III

*Relationship between maternal and cord blood haemoglobin*

| Maternal hb. g./per 100 ml. | 6– | 7– | 8– | 9– | 10– | 11– | 12– | 13– | 14– |
|---|---|---|---|---|---|---|---|---|---|
| Nos. | 3 | 15 | 50 | 81 | 114 | 71 | 66 | 22 | 10 |
| Mean cord blood hb. g./ per 100 ml. | 12·9 ±2·9 | 13·4 ±1·3 | 12·8 ±1·7 | 13·1 ±1·8 | 13·4 ±1·6 | 13·1 ±1·4 | 14·0 ±1·4 | 14·7 ±1·2 | 14·9 ±1·3 |

level of maternal serum iron. Maternal haemoglobin levels and cord blood haemoglobin levels have no constant relationship to the level of maternal serum iron. The mean maternal serum iron level for the low birthweight babies was 88±42 (SD) μg./dl.; and that for the high birthweight babies was 82±41 μg./dl.

### *Maternal haemoglobin level and cord blood haemoglobin level*

Table III shows the relationship between maternal and cord blood haemoglobin. At maternal haemoglobin levels below 12 g./dl. the effect on the cord blood was statistically insignificant as cord blood levels remained around 13 g./dl. However, when maternal haemoglobin was 12 g./dl. and over the cord blood haemoglobin rose to 14 g./dl. and above. The difference in cord haemoglobin levels between the two groups of maternal haemoglobin levels was statistically significant at the 5 per cent level. Overall, for the 432 mothers, the mean maternal haemoglobin level was 10·6±1·7 g./dl. whilst that of the cord blood was 13·4±1·7 g./dl.

## DISCUSSION

Kagwa-Nyanzi (1972) in Uganda found no correlation between maternal anaemia and the baby's haemoglobin status. She concluded that normal infants are parasitic *in utero* irrespective of the maternal anaemic state, and that the African full term infant is not anaemic. Our results showed similarly that despite maternal anaemia the infant was comparatively non-anaemic. However, compared to international levels for infants at birth these infants were in fact anaemic. Taking non-anaemic mothers with haemoglobin levels above 12 g./dl., the cord blood levels tended towards normal international levels. Nelson (1969) states that the mean cord blood haemoglobin is 16·8 g./dl. for Caucasians. Bwibo (1971) in a review article says that an infant born to an iron-deficient mother has less iron stores even if born at term and thus is in the first stage of the iron deficiency state. We did not do bone-marrow examinations in infants to confirm this statement.

Our study showed a direct relation between maternal and cord blood haemoglobin but this relation was only demonstrable when one separated the anaemic from non-anaemic mothers; and we demonstrated a direct linear relationship between maternal and cord serum iron levels. The fetus, being an efficient parasite, extracted optimum amounts of iron in direct proportion to the levels available in the mother in such a way that even in very anaemic mothers it extracted enough iron to raise its haemoglobin levels to about 13 g./dl.

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# CARBOHYDRATE-INDUCED LIPOGENESIS IN THE HUMAN PLACENTA OF NORMAL AND DIABETIC PREGNANCIES

BY

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## Summary

Using *in vitro* incubation, human placental slices have been shown to synthesize lipid from $^{14}$C fructose, but to a lesser extent than from $^{14}$C glucose. Diabetic placentae did not incorporate more of either sugar into lipid than did placentae from normal pregnancies; nor did insulin in the incubation medium enhance lipogenesis. There was a correlation between the extent of incorporation of $^{14}$C fructose and $^{14}$C glucose into triglyceride, suggesting a linked enzyme system for phosphorylation of the two hexoses.

GLUCOSE has been shown to form lipid in both the human placenta (Szabo and Grimaldi, 1970) and fetus (Coltart, 1972). Fructose alone is also known to be lipogenic (Kuo *et al.*, 1967; Zakim, 1972) as is the fructose of sucrose (Coltart and Crossley, 1970), a disaccharide in which fructose and glucose are present in equimolar amounts. Since sucrose is an important dietary constituent of most pregnant women, the following study was designed to investigate fructose as a possible substrate for lipid synthesis in the placenta of normal and diabetic mothers.

## PATIENTS AND METHODS

Placentae were obtained from 15 patients. Eleven had had uncomplicated pregnancies and four were well-controlled insulin-dependent diabetics. None had eaten within 12 hours of delivery, but ten were receiving glucose infusions during labour.

At delivery the placenta was transferred immediately to the laboratory in ice-cold isotonic saline solution. Serial slices weighing between 100 and 250 mg. were obtained from the centre of a healthy cotyledon (confirmed subsequently on histological examination), such that both fetal and maternal surfaces were excluded. The slices were incubated for two hours at 37 °C. in 3 ml. of Krebs-Ringer bicarbonate buffer (previously gassed with 95 per cent oxygen and 5 per cent carbon dioxide) containing 100 mg. per 100 ml. glucose or fructose. To each glucose-containing flask was added 2 $\mu$ Ci of $^{14}$C glucose and to each fructose-containing flask 2 $\mu$ Ci of $^{14}$C fructose. To alternate glucose and fructose flasks was added 0·1 units of soluble insulin in 1 per cent bovine albumin. Maximum time interval between delivery of the placenta and starting incubation was 30 minutes.

Following incubation the placental tissue was rinsed in distilled water and macerated with chloroform/methanol:2/1. Lipid extraction was undertaken according to the method of Folch *et al.* (1954). Separation of the various lipid fractions was carried out on heat-activated silica plates (0·5 g. kieselgel/ml.) using thin layer chromatography in a system containing petroleum spirit, diethyl ether and glacial acetic acid in the ratio of 82:18:1 by volume (Schlierf and Wood, 1965).

After removal of phospholipid on the baseline, the free and esterified cholesterol and triglyceride fractions were rendered visible with iodine and identified by comparison with a simultaneously run pure lipid standard of known composition. The phospholipids were re-extracted with methanol and the remaining fractions with diethyl ether. Radioactivity in the lipid fractions was measured using a scintillation counter, with toluene containing 5 g. of 2,5-diphenyloxazole (PPO) and 100 mg. of 1,4-di [2, (5-phenyloxazolyl)] benzine (POPOP) per litre as the scintillant. Quenching was corrected by the external standard ratio method.

The results were derived from duplicate incubations, radioactivity being expressed as disintegrations per minute (dpm) per gram wet weight of tissue.

## RESULTS

Table I shows the level of incorporation of $^{14}$C labelled glucose and fructose into placental lipid of normal and diabetic pregnancies. Highest counts were found in the triglyceride and phospholipid fractions. Placentae from diabetic pregnancies did not incorporate either hexose into lipid to any greater extent than did those from normal pregnancies, nor did the presence of insulin in the incubation medium enhance lipogenesis. There was, however, significantly more incorporation of labelled glucose than fructose into the triglyceride ($P<0\cdot025$) and free cholesterol ($P<0\cdot001$) fractions.

In Figure 1 the counts in the triglyceride fraction from $^{14}$C glucose are plotted against the corresponding counts from $^{14}$C fructose. There is a significant correlation between the extent of incorporation of the two sugars ($r = 0\cdot885$ with 13 degrees of freedom; $P<0\cdot01$).

## DISCUSSION

The results indicate that the human placenta is capable of synthesizing lipid from fructose,

TABLE I

*Incorporation of* $^{14}$C *glucose and* $^{14}$C *fructose into placental lipid fraction (expressed as disintegrations per minute/g. wet tissue) for normal and diabetic pregnancies, with and without soluble insulin in the incubation medium. Mean values* (±SEM) *derived from duplicate incubations of 11 normal and 4 diabetic placentae*

| | | Incorporation of $^{14}$C glucose or fructose (dpm./g. wet weight tissue) | | | |
|---|---|---|---|---|---|
| | | Glucose | Glucose+ Insulin | Fructose | Fructose+ Insulin |
| *Cholesterol esters* | | | | | |
| Normal | Mean | 400 | 502 | 619 | 332 |
| | SEM | 114 | 121 | 151 | 91 |
| Diabetic | Mean | 488 | 338 | 437 | 371 |
| | SEM | 115 | 85 | 93 | 94 |
| *Triglyceride* | | | | | |
| Normal | Mean | 7274 | 7628 | 4007 | 3496 |
| | SEM | 2438 | 2206 | 1096 | 1045 |
| Diabetic | Mean | 10794 | 9995 | 6366 | 4858 |
| | SEM | 4663 | 4609 | 2135 | 1052 |
| *Free Cholesterol* | | | | | |
| Normal | Mean | 3078 | 2648 | 1658 | 1534 |
| | SEM | 395 | 338 | 181 | 166 |
| Diabetic | Mean | 2776 | 2382 | 2412 | 1749 |
| | SEM | 472 | 134 | 680 | 758 |
| *Phospholipids* | | | | | |
| Normal | Mean | 5617 | 9594 | 3541 | 4660 |
| | SEM | 1724 | 4340 | 1550 | 1527 |
| Diabetic | Mean | 8641 | 7479 | 8371 | 6645 |
| | SEM | 3571 | 4328 | 3864 | 3328 |

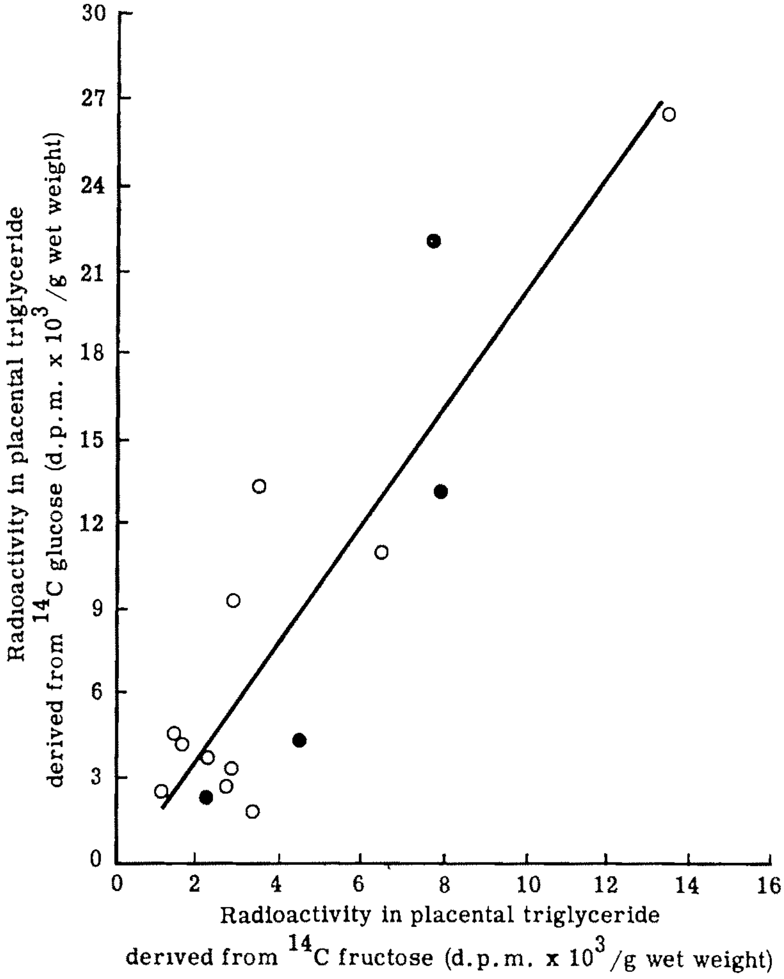

FIG. 1

Mean levels of radioactivity (disintegrations per minute/g. wet weight of placenta) in placental triglyceride following incubation with $^{14}$C fructose plotted against the corresponding mean level of radioactivity from $^{14}$C glucose ($r = 0{\cdot}885$; $P<0{\cdot}01$). Open circles represent normal placentae. Closed circles represent diabetic placentae.

The overall lipogenic capacity of fructose, however, is less than that of glucose, due to the significant difference between the glucose and fructose with respect to the incorporation of the two hexoses into triglyceride and free cholesterol. These findings are in contrast to those obtained in studies on human liver, where fructose incorporation into triglyceride is greater than that of glucose (Kuo *et al.*, 1967; Zakim, 1972).

Also of note is the relatively high percentage of total counts in the free cholesterol fraction, an observation in keeping with previously reported levels of glucose incorporation into free cholesterol in human fetal liver (Coltart, 1972). Such levels are much greater than those in adult liver (Kuo *et al.*, 1967). This finding could be accounted for by the cholesterol "pool" size in fetal tissue being proportionately smaller than that in the adult, although it is tempting to speculate that the higher counts are indicative of more active cholesterol synthesis, underlying the importance of cholesterol as a

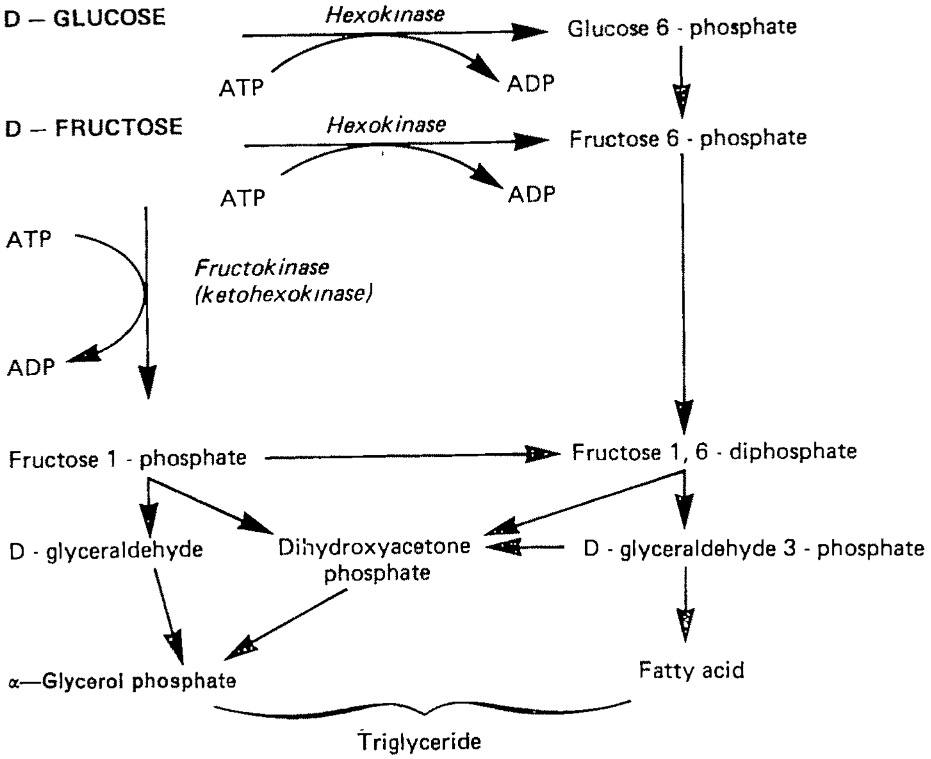

FIG. 2

The metabolism of fructose and glucose into triglyceride, showing some of the major intermediaries.

precursor of steroid hormones in the feto-placental unit.

Insulin has been shown previously to have no effect on lipogenesis in the human placenta (Roux and Green, 1967; Szabo and Grimaldi, 1970) and our results conform with these observations. Reasons for such findings with respect to glucose include the presence of an insulinase in the placenta (Goodner and Frienkel, 1959), and the high levels of placental lactogen, a hormone which is known to have lipolytic (Turtle and Kipnis, 1967; Genazzani *et al.*, 1969) and hence anti-insulin properties.

There is good evidence to suppose that raised levels of plasma-free fatty acid (FFA) enhance hepatic triglyceride synthesis (Nikkilä, 1973; Adams *et al.*, 1974). In pregnant women levels of FFA are raised (Picard *et al.*, 1968) particularly if the pregnancy is complicated by diabetes (Molsted-Pedersen *et al.*, 1972). If one assumes both hepatic and placental triglyceride to be derived from the esterification of α-glycerophosphate by FFA it is probable that the apparently "normal" level of triglyceride counts in the diabetic placentae reflect satisfactory control of the diabetes.

The correlation between the extent of incorporation of glucose and fructose into placental triglyceride suggests that hexokinase (EC.2.7.1.1.) may be responsible for phosphorylation of both hexoses (Fig. 2). Nevertheless, it is feasible to postulate two separate pathways, with fructose undergoing phosphorylation via fructokinase (ketohexokinase EC.2.7.1.12) to fructose 1-phosphate, and in which the activity of fructokinase and hexokinase are in some way linked. Hexokinase is known to have a lower $K_m$ for glucose than fructose indicating greater affinity for glucose than fructose on a molar basis, and this could account for the higher triglyceride counts with glucose. Studies into the phosphorylation of fructose and glucose by the human placenta are currently in progress.

It is tempting to speculate as to the fate of triglyceride synthesized in the placenta, since it is thought that the intact triglyceride molecule does not cross the placenta (Robertson and Sprecher, 1968). There is, however, good evidence that the feto-maternal barrier is permeable to certain fatty acids (Szabo *et al.*, 1969; Dancis *et al.*, 1973) and if one assumes the placenta contains triglyceride lipase for hydrolysis of triglyceride to glycerol and fatty acid, it is feasible to postulate that the placenta acts as a short-term reservoir for fatty acids intended for the fetus. Indirect evidence to support this concept stems from the apparent ineffectiveness of insulin in the placenta with regard to promoting lipogenesis by the esterification of α-glycerophosphate and free fatty acid. In adipose tissue insulin enhances lipogenesis (Flatt, 1970). It also inhibits lipolysis possibly by decreasing intracellular levels of 3′, 5′-cyclic AMP (Butcher *et al.*, 1966; Siddle and Hales, 1974). Thus was the action of insulin on lipolysis to be as ineffective as it has been shown to be on lipogenesis, the stage is set for a continuous net release of fatty acids into the fetal circulation.

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# MASSIVE SUBCHORIAL THROMBOHAEMATOMA (BREUS' MOLE)

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## Summary

Ten placentae with massive subchorionic thrombohaematomas are reported. This lesion, which is composed of maternal clotted blood, separates the chorionic plate of the placenta from the villous chorion and is traversed by the main cotyledonary branches of the fetal circulation. In some instances it dissects into the layers of the chorionic plate. In anatomical terms it is identical to the lesion which has been described in early pregnancy under the title of Breus' mole. Previous reports suggested that the lesion was confined to early pregnancies and was a *consequence* of fetal death. In our series all ten placentae were from pregnancies of at least 25 weeks' maturity and six were more than 30 weeks. Seven infants were liveborn and three survived the neonatal period. Differential diagnosis is discussed along with aetiology, which is still obscure although earlier theories implicating fetal death may be discounted.

SUBCHORIAL fibrin deposits, presumably the end results of small thromboses, are an almost constant finding in term placentae. Very occasionally, however, a massive thrombohaematoma develops in this site. This was first described by Breus (1892) in association with missed abortions and subsequently referred to as Breus' mole. The purpose of this paper is to record ten further examples occurring in the latter half of pregnancy and to attempt clarification of terminology and theories about aetiology in the light of new evidence.

## MATERIAL

A total of 19 000 placentae was personally studied. A placenta was recorded as having massive subchorial thrombohaematoma when coagulated blood over 1 cm. thick separated the villous chorion from the membranous chorionic plate over much of its area. One of us (D.R.S.) found seven examples in 14 000 placentae in the United States, while the other (J.S.S.) found three examples in 5000 placentae in the United Kingdom. The overall incidence of thrombohaematoma was thus 0·53 per thousand.

The salient clinical and pathological features are given in Table I. Four placentae were from deliveries at 25 to 28 weeks, but the remainder were from pregnancies which terminated after 31 weeks. Three of the fetuses survived, four died in the neonatal period, two were stillborn, and one was dead at spontaneous abortion. The

TABLE I

*Details of ten patients with subchorionic thrombohaematoma*

| Patient No. (and author) | Maternal age | Gravidity | Gestation (weeks) | Clinical notes | Placental description |
|---|---|---|---|---|---|
| 1. (J.S.S.) | 21 | 1 | 25 | Vaginal bleeding; uterus small for dates; late abortion. | 6·0 × 6·0 × 3·0 cm. The chorionic plate had a morula-like appearance and was separated from a thin layer of villous tissue by a mass of clotted blood which was fresh and which extended throughout the subchorial space (Figs. 1 and 2). |
| 2. (J.S.S.) | 33 | 2 | 40+ | Induction for postmaturity by amniotomy; fetal heart stopped immediately afterwards; 3610g stillbirth. | 863g. 18·7 × 14·0 × 1·7 cm. Massive, central, subchorial haematoma which disrupted the decidual surface over an area of 6·0 × 5·0 cm. or 11·5 per cent of surface area (Figs. 3 and 4). |
| 3. (J.S.S.) | 34 | 3 | 34 | Syphilis diagnosed at 25 weeks; treated with penicillin; abdominal pain, slight vaginal bleeding, uterus tense at 31 weeks; hypertension at 33 weeks; 1317g female infant died at five days following respiratory distress. | 880g., 14·0 × 16·5 × 7·5 cm. Haematoma 6·5 cm. thick, weight roughly 610g. The mass extended throughout the subchorial space. Major fetal vessels could be seen glistening through the grossly distended membranous surface; on section after 10 per cent formalin fixing for two months major fetal vessels were evident running through 6·5 cm. of clot to the villous chorion; these vessels appeared to contribute to the tuberous appearance; Levaditi staining negative for spirochaetes (Fig. 5). |
| 4. (D.R.S.) | 20 | 7 | 25 | Rupture of membranes at 23 weeks treated with penicillin; 825g female infant lived nine hours, maternal hypofibrinogenaemia. | 245g. 11·0 × 14·0 × 3·0 cm. Complete placenta extrachorialis of circumvallate type. A dense, gelatinous, yellowed plaque 1·0 to 1·6 cm. thick, was subjacent to the entire chorionic plate (Fig. 6). |
| 5. (D.R.S.) | 17 | 1 | 28 | Maternal haematocrit 17 per cent; 1040g male stillborn with maceration consistent with death 24 to 36 hours previously. | 840g. 20·0 × 16·0 × 3·0 cm. A thick, bulbous, fresh multilobulated (tuberous) haematoma beneath the entire chorion (Fig. 7). |
| 6. (D.R.S.) | 30 | 7 | 40 | Mild hypertension; 3150g infant; alive and well. | 502g. 18·0 × 16·0 × 2·0 cm. A surface sac measuring 7·5 × 4·0 × 2·5 cm. was irregular grey-white with a red, protuberant mass 5·0 × 2·0 × 1·0 cm.—10 per cent of the surface area. (Similar to Patient No. 7.) |
| 7. (D.R.S.) | 30 | 4 | 36 | Moderately severe diabetes mellitus; infant alive and well. | 620g. 15·5 × 15·5 × 4·0 cm. A degenerate, mixed, cystic chorionic haematoma 6·0 × 6·5 × 4·0 cm.; 85g. This occupied about 17 per cent of the chorionic plate (Figs. 8 and 9). |
| 8. (D.R.S.) | 20 | 1 | 31 | Mild diabetes controlled by diet; epilepsy; 2955g male infant; alive and well. | 485g. 16·0 × 17·0 × 2·5 cm. Multiple subchorial thrombohaematomas covered one-third of the surface. |
| 9. (D.R.S.) | 16 | 1 | 26 | Normal until premature labour. 560g female infant survived 24 hours. Autopsy; atelectasis; omphalitis; immaturity. | 150g. 12·5 × 10·0 × 1·0 cm. protuberant thrombohaematoma 7 × 10 × 2 cm.; 55 per cent of surface area; dark tan, soft, with red zone at base. On sectioning this was a laminated thrombus, the oldest parts being directly subjacent to the chorion. |
| 10. (D.R.S.) | 28 | 8 | 34 | Severe hypertension treated by thiazides; massive vaginal haemorrhage followed by rapid labour; living 2325g infant died at 20 hours with haemorrhagic lesions and thrombocytopenia possibly related to thiazides (Shanklin, 1969). | 450g. 10·0 × 14·0 × 2·5 cm.; massive 7 cm. diameter almost spherical central thrombohaematoma; 20 per cent of surface area; no laminations, uniform fresh red colour; no apparent compression of lateral cotyledons but some bulging of surface. |

TABLE II
*Interrelation between size and age of lesion*

| Size of lesion (per cent of surface area) | Age of lesion (Patient No.) | | | Total No. of patients |
|---|---|---|---|---|
| | Fresh | Mixed | Old | |
| 10 to 20 per cent | 2, 10 | 6, 7 | – | 4 |
| 30 to 40 per cent | 8, 9 | – | – | 2 |
| 100 per cent | 1, 5 | 3 | 4 | 4 |
| Total number of patients | 6 | 3 | 1 | 10 |

gravidity order ranged from one to eight and only one mother had previously aborted. One mother had been treated for syphilis and also developed late pregnancy hypertension; two others had hypertension, one severe and one mild; two had diabetes which was not severe; one had epilepsy, and another severe anaemia.

Table II shows the age of the process and the size of the lesion. These appear independent, even though the one definitely old lesion involved the total area of the chorionic plate (Patient No. 4). The appearance of six of the placentae with thrombohaematomas is shown in Figures 1 to 9.

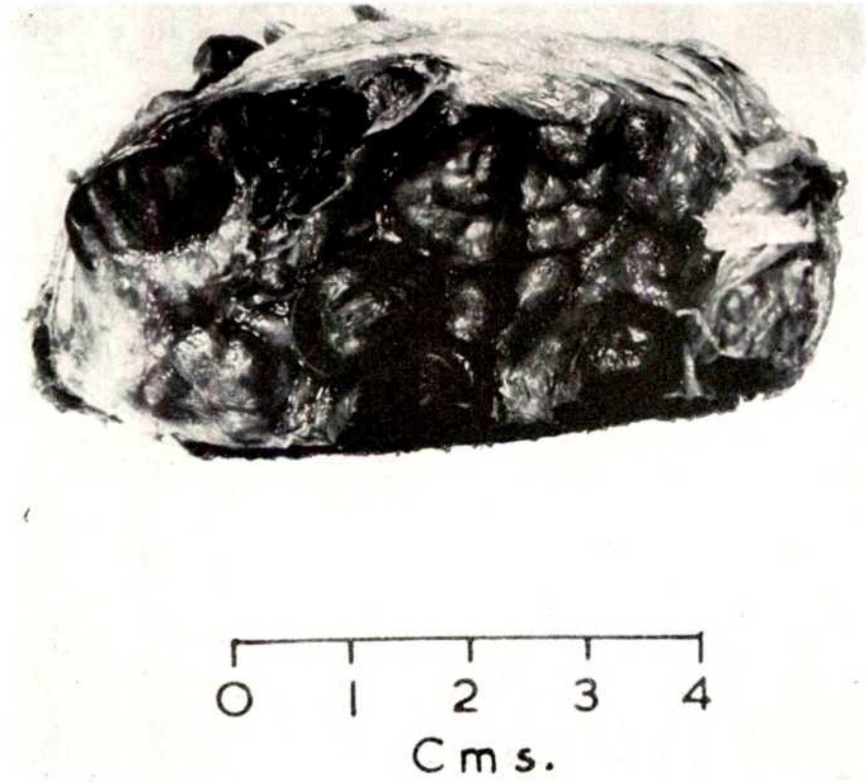

FIG. 1
Chorionic surface of placenta from Patient No. 1, showing morula appearance with multiple tuberous projections of varied size. The fresh red clot, which appears dark in the photograph, indicates the recent origin of the thrombohaematoma.

FIG. 2

Transverse section of placenta from Patient No. 1, showing a suggestion of layering of the fresh thrombus parallel to the chorionic surface.

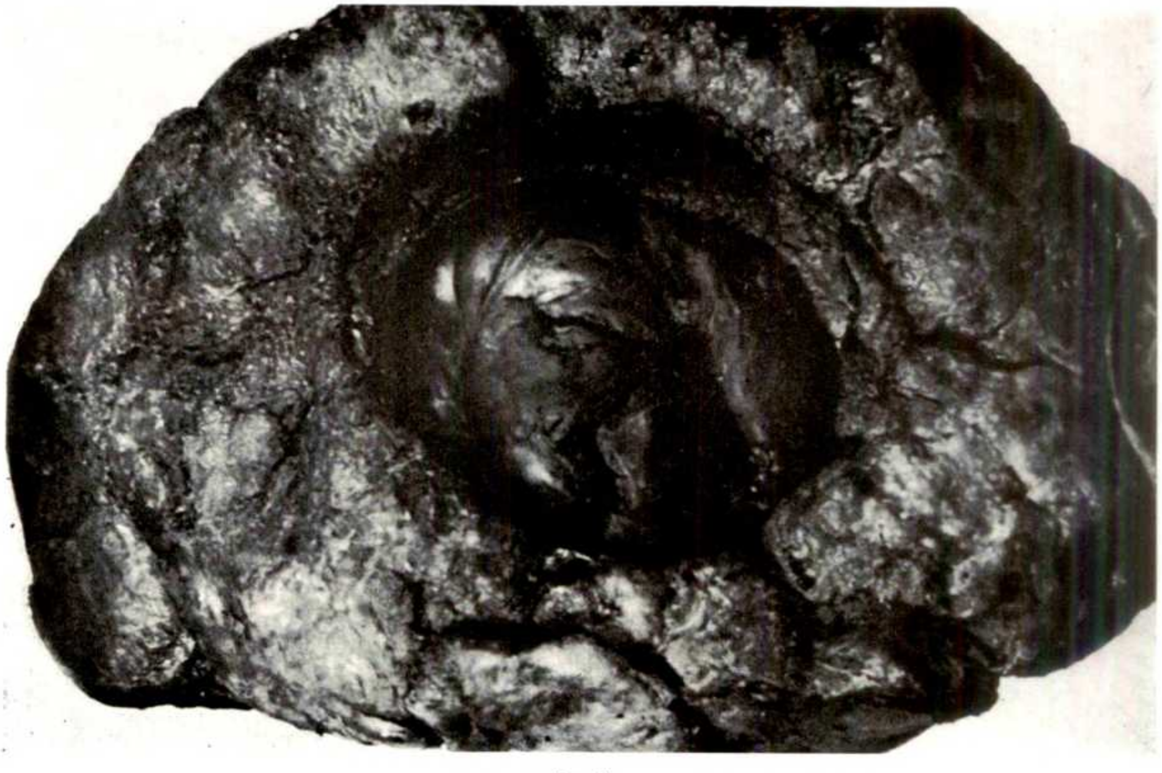

FIG. 3

Decidual view of placenta from Patient No. 2, showing lobulated, massive transplacental thrombohaematoma. The subchorial origin of the lesion is seen in the transverse section (Fig. 4).

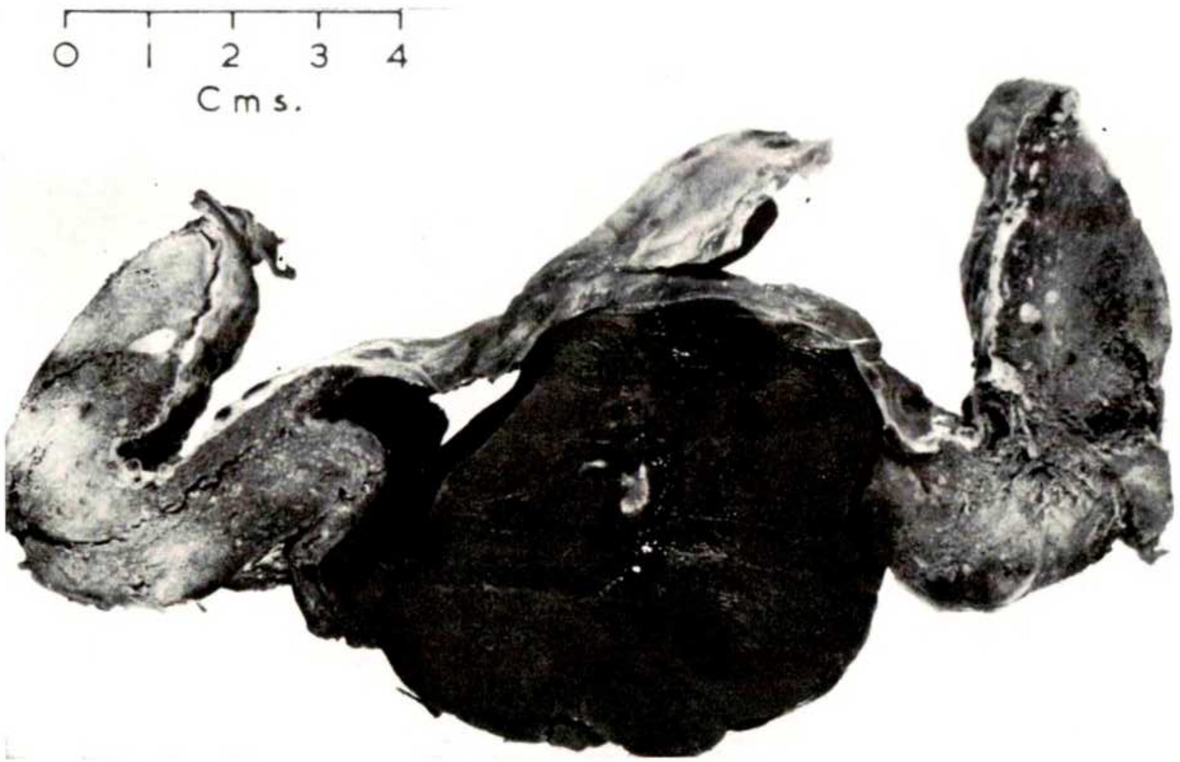

FIG. 4

Transverse section of placenta from Patient No. 2, showing that both the subchorionic and the decidual aspects of the mass are extensive and up to four times the thickness of the placenta itself. Note the lack of compression or displacement of cotyledons.

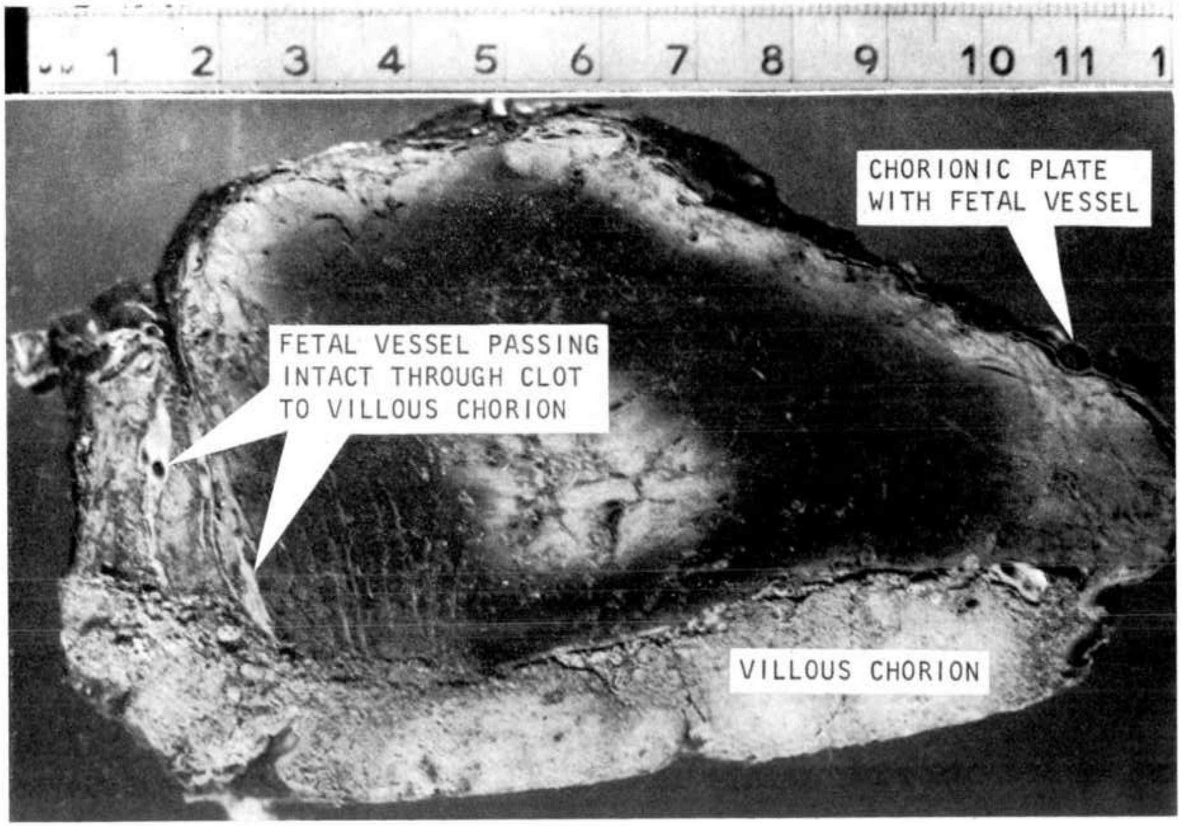
1 2 3 4 5 6 7 8 9 10 11 1
CHORIONIC PLATE WITH FETAL VESSEL
FETAL VESSEL PASSING INTACT THROUGH CLOT TO VILLOUS CHORION
VILLOUS CHORION

FIG. 5

Placenta from Patient No. 3, showing a massive thrombohaematoma which accounted for two-thirds of the placental weight with fetal vessels passing intact through the lesion from chorionic plate to villous chorion. The fetus was born alive despite the complete separation of the chorionic plate. It can be seen that at the site of major fetal vessels the chorionic plate tends to be held down giving the lesion a tuberous appearance.

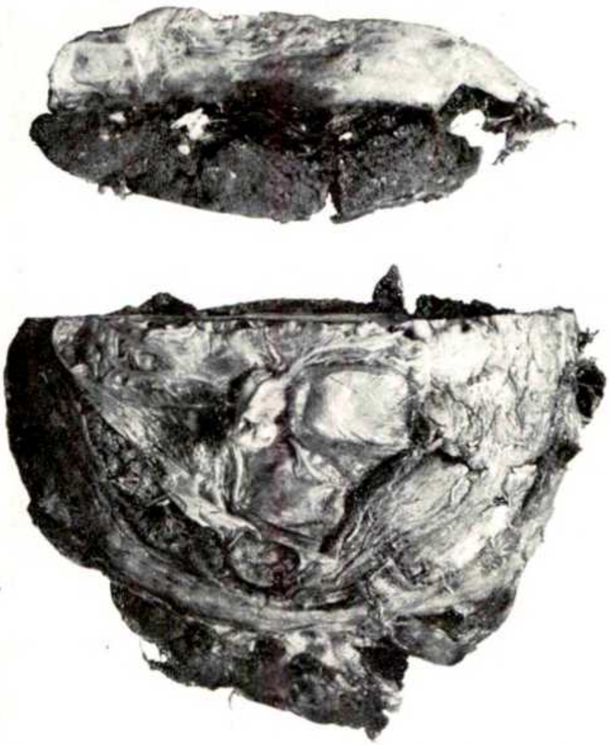

Fig. 6

Placenta from Patient No. 4 showing massive, old subchorionic thrombohaematoma occupying the entire subchorionic space. The lesion accounted for about 50 per cent of the weight of the placenta. Infant born alive and survived nine hours. Some coarse trabeculation suggested the possibility of the lesion being laid down in stages, but there were no fresh components.

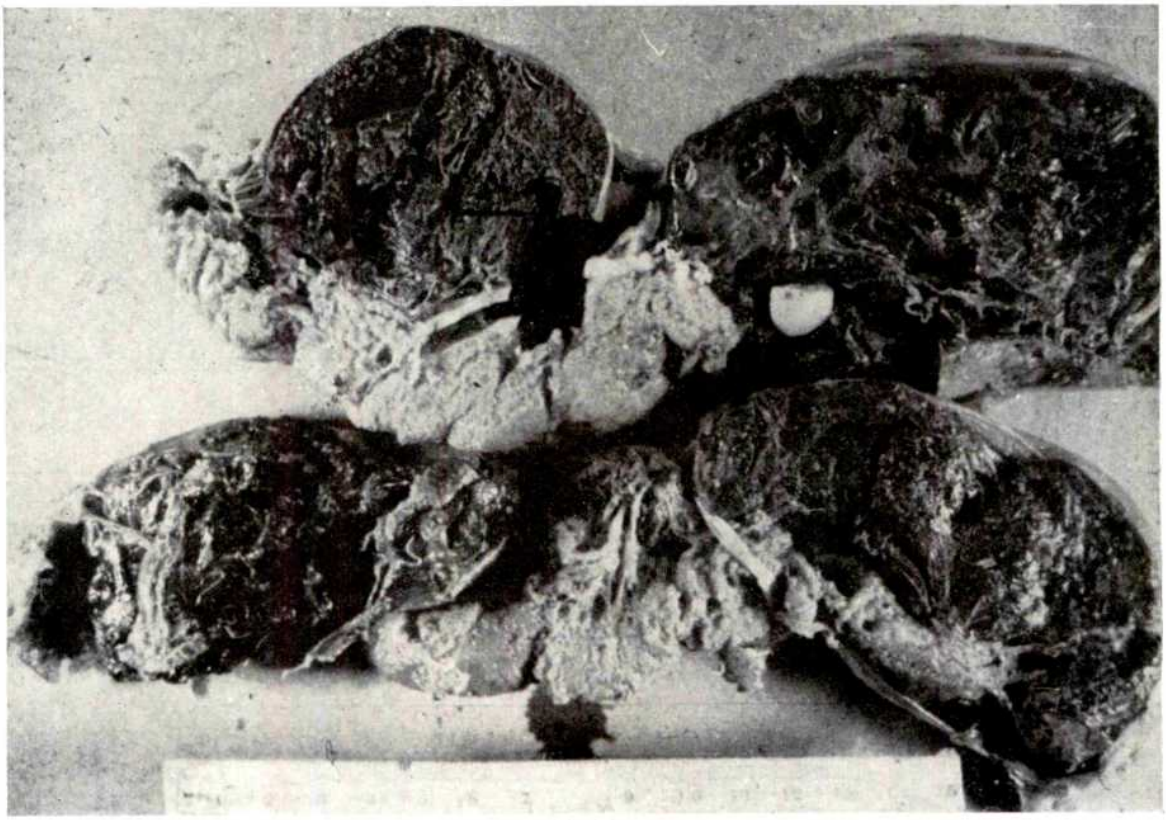

Fig. 7

Placenta from Patient No. 5 showing a thick, bulbous or tuberous, fresh haematoma beneath the entire chorion. The striae in the clot are produced by laminae and fetal blood vessels.

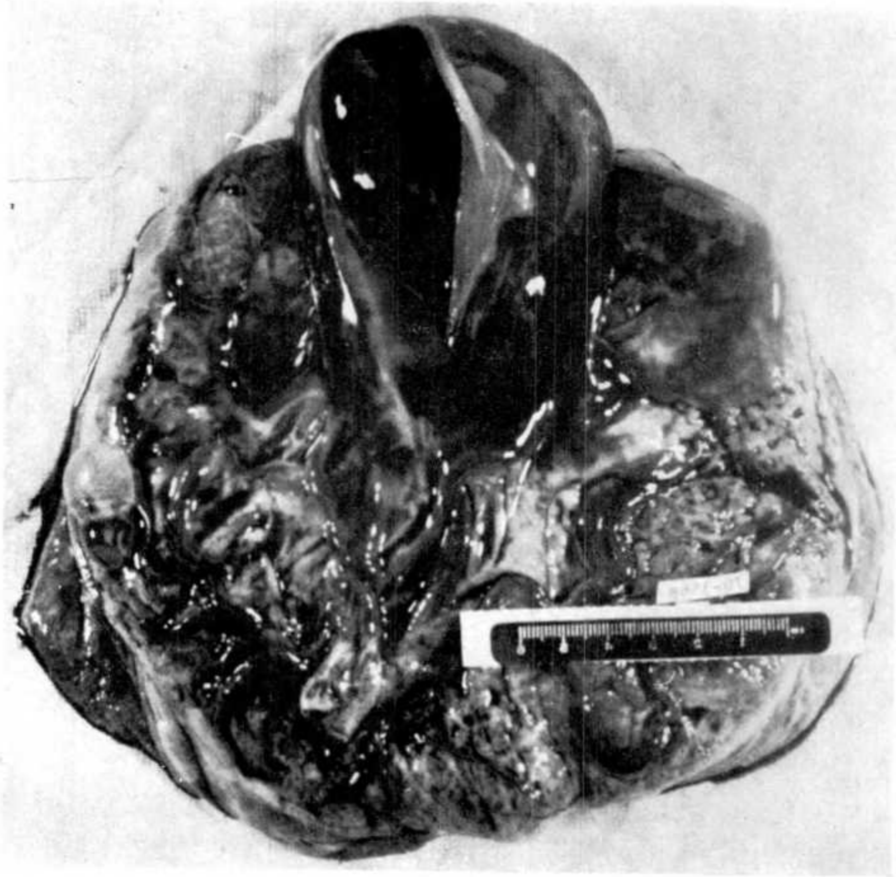

FIG. 8

Placenta from Patient No. 7 showing a small, discrete and fresh thrombohaematoma. The amnion has been reflected across part of the thrombotic lesion. Microscopically this was a fused, thinned amniochorion, preserving the site of the basic lesion as subchorionic. Note the smaller, lateral cyst-like structures. These are similar, but without clotted blood, suggesting a primary defect in the chorio-amnion possibly accentuated by flow pressure.

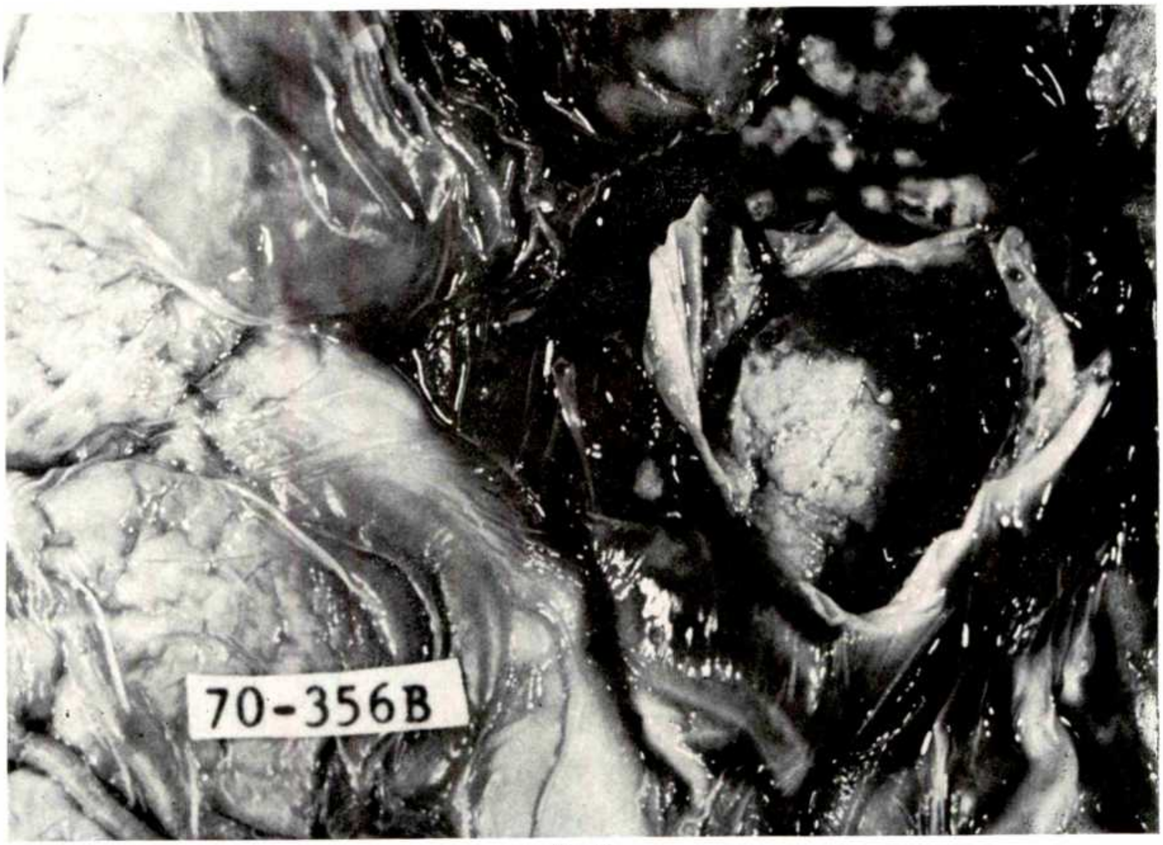

FIG. 9

Close up view of placenta from Patient No. 7, showing an old, shaggy fibrin pad at the base of the lesion. Near the specimen label there is an extensive subchorionic deposition of fibrin without haematoma formation.

## DISCUSSION

The original description of the lesion gave rise to the designation 'Breus' mole'. These moles consist of masses of blood thrombus with ultimate fibrin formation and the term *mole*, in this context, probably comes from Latin through French 'moles' meaning 'a mass'. The British Medical Dictionary defines thrombus as 'a blood clot *formed in* and *remaining in* a blood vessel'. Because the human placenta is haemochorial, the trophoblast-lined intervillous space is the equivalent of a vessel. Since these lesions occasionally dissect beyond the usual confines of the intervillous space between the layers of the chorionic plate it therefore seems appropriate to add the term 'haematoma' after 'thrombus'. Our preferred pathogenetic term 'thrombohaematoma' is a modification of 'tuberous hematoma', which was suggested by Reid (1962).

The key to gross recognition of this lesion, as emphasized by Ashworth and Stouffer (1956), is that the chorionic plate, with its fetal vessels running in a branching manner from the umbilical insertion, is stripped away from the chorionic villous tissue by the thrombohaematoma. The clot is formed principally, if not entirely, by maternal blood.

Pathologically important thrombohaematoma formation may occur at several sites in the human placenta, but the most important clinically is the decidual plate of uterine attachment. Large lesions in this site are termed "abruptio placentae" and these have an incidence of about 0·85 per cent (Shanklin, 1972).

It is important to differentiate Breus' mole (subchorial thrombohaematoma) from other types of placental mole. There is little likelihood of confusion with hydatidiform mole. Carneous, fleshy or decidual mole (missed abortion) represents a more difficult problem in differentiation. Benirschke and Driscoll (1967) describe Breus' mole as a 'morphologic variant of missed abortion'. The usual clinical presentation of missed abortion may, of course, be present with an early, massive subchorial thrombohaematoma (Breus' mole) but it is more likely, as Linthwaite (1963) recorded, that the chorionic gonadotrophin level will remain higher with the Breus thrombohaematoma than with the decidual mole as in the latter situation the blood supply of the chorionic tissue is completely severed. The essential distinction is that the blood mass is on the maternal (decidual) side of the chorionic villous tissue in the carneous or fleshy mole whereas in Breus' mole the thrombohaematoma is on the fetal side directly beneath, or within the layers of, the chorionic plate.

Another lesion which requires differentiation is subamniotic haematoma—a haematoma between amnion and chorion. It has an incidence of about 1 in 400 (Scott and Shanklin, 1973). Unlike the decidual or subchorionic thrombohaematoma, the lesion is definitely extravascular and therefore is appropriately called simply a "haematoma"; also unlike the other types considered it is composed of fetal blood. An artefactual subamniotic haematoma is frequently produced by cord traction.

Virtually every placenta has a fine layer of fibrin deposited on the villous side of the chorionic plate while approximately 20 per cent show discrete fibrin laminae (Shanklin, 1959). Ashworth and Stouffer (1956) observed that such subchorionic fibrin plaques may produce separation between the membranous chorionic plate and the chorionic villi and claimed that there was a resultant defect in the syncytial lining, providing a site for thrombosis. The fibrin plaques usually appear old in relation to thrombi found elsewhere in the placenta. Whether these lesions, which usually measure 2 to 3 cm. in diameter, are related to the massive thrombohaematomas we describe is uncertain.

The incidence of these minor subchorionic thrombohaematomas was about 1 in 1200 in our series but the figure depends very much on the individual observer.

Hertig and Sheldon (1943) describe Breus' mole as an "intraplacental hematoma" and recorded an incidence of 19 per 1000. This is much higher than our incidence and that of other reports. It is true that a subchorial thrombohaematoma may *become* an intraplacental haematoma (see our Patients 2 and 10). It seems appropriate, however, to classify as Breus' mole only those intraplacental lesions which have obviously arisen from the subchorionic region and involve extensive separation of the chorionic plate with protuberant masses of thrombus beneath it (Linthwaite, 1963).

The occurrence of massive subchorial thrombohaematomas in relatively mature placentae in our series contrasts with the impression given in most reports that they are mainly, if not exclusively, found in association with abortion. This discrepancy may be partly explained by the fact that these examples were observed during sequential study of series of placentae and when such systematic studies are performed all mature placentae tend to come for examination.

The original concept of Breus (1892) was that fetal death occurred but that the placental membranes continued to grow and redundant folds of the chorion became filled with blood which clotted. Gottschalk (1899*a* and 1899*b*), Taussig (1904) and Benirschke and Driscoll (1967) accepted this view. The alternative explanation that the thrombohaematoma formation *caused* fetal death was put forward by Neumann (1897), Davidsohn (1902), Hart (1902), Hutchinson (1906), Reid (1962), Linthwaite (1963), Thomas (1964) and Torpin (1960).

Our observation of massive lesions of this type associated with live births in seven out of ten instances is strongly against the view of Breus that fetal death is primary rather than secondary; rather it seems that fetal death will occur if the placental circulation is greatly interfered with by the thrombohaematoma. Information concerning a lesion of this type in an *in situ* placenta illustrated by Villee (1960) also supports our observation. The late J. D. Boyd (1967, personal communication), stated that the fetus in this case, which had a crown-rump length of 210 mm. (not 145 as quoted by Villee) and was thought to be of about 20 weeks gestation, showed no maceration and was well nourished. In his view the fetus had survived the formation of the placental lesion.

The tuberous or nodular character of the chorionic plate in the cases with relatively fresh thrombi was probably the consequence of a thrombohaematoma produced by the perfusion pressure as with a dissecting aneurysm—in this case dissecting into the membranous chorion; the major chorionic vessels and the intercotyledonary septa limit expansion, producing the tuberous appearance. Hörmann (1958) in fact chose the designation "mola aneurysmatica".

Patient No. 5 closely resembles those described by Breus. Delivery occurred at 28 weeks. The tension on fetal vessels, and possibly compression of the parenchyma of the placenta, compromised the fetus irreversibly.

Whether the "tuberous" variety of lesion can convert with time to the "flat", compressed apparently old type represented by Patient No. 4 is uncertain. The fact that the lesion has hitherto been recorded in association with first trimester abortions could be regarded as evidence against its persistence to late pregnancy and then presenting in a "flat" form. Patient No. 1 had both "tuberous" and "flat" qualities but as Figures 1 and 2 show, the tuberosities were not as large as those in Patient No. 5 (Fig. 7).

In relation to the duration of retention of dead fetuses, 77 per cent are delivered within 72 hours and 92 per cent within 168 hours (Shanklin, 1964). However, these figures apply to dead fetuses of all types and the lesion being considered has special characteristics which make a different pattern of behaviour possible. Although the baby dies, the chorionic tissue is still attached to the decidua and receiving its full blood supply so that chorionic gonadotrophin production may be continuing. Immediate abortion may therefore be unlikely to occur and the pregnancy may progress for significantly longer than is usual after fetal death. This is in contrast to massive *decidual* haematoma (abruption) where expulsion usually follows very promptly after fetal death. This aspect was discussed in detail by Linthwaite (1963). In Patient No. 3 it is fairly certain that the massive thrombohaematoma which formed 66 per cent of the total placental weight developed three weeks before delivery when the mother had abdominal pain, slight vaginal bleeding and uterine hypertonus. In Patient No. 4 the infant was also born alive. The uniformity of compaction and liquefaction of this thrombohaematoma suggests the lesion occurred about ten days before delivery; it could not have been longer because the placenta would have grown beyond the haematoma's limits. The concurrence of both old and fresh lesions indicates, however, that progression and recurrence are possible. In Patients No. 6, 7 and 9 there were localized lesions and a fresh terminal or possibly intrapartum haematoma. Evidence that small lesions progress suggests the possibility that the massive lesions simply incorporate the original one and obscure any "stage" effect. Two of the lesions with apparent progression protruded into the amniotic cavity; the others spread along the chorionic plate. It is possible that a focal weakness in the chorio-amnion would encourage protrusion in the direction of the amnion.

In Patients No. 2 and No. 10 the haematoma was so massive it extended to the decidual surface becoming in effect a *trans*placental thrombohaematoma. In Patient No. 10 platelet-toxic drugs were thought to be a factor in pathogenesis —both mother and baby had thrombocytopenia. Apparently a dissecting type of leak had occurred between the layers of the chorionic plate with ultimate thrombohaematoma formation. There was no history of thrombocytopenia in Patient No. 2. In neither case was there significant compression of adjacent placental tissue.

The aetiology of Breus' mole remains almost as obscure as at the time of earlier reports except that the notion that it is always a *consequence* of fetal death can now be excluded. It is probable that the circulatory rate in the subchorionic region is slower than in other zones of the placenta and thus makes extensive thrombosis there more likely (Linthwaite, 1963). Although the range of maternal illness in this series seems considerable no clear pattern emerges, and a high proportion of patients in specialist obstetric units, such as ours, do have medical complications. Only von der Ahe (1965) has reported a recurrence in a subsequent pregnancy. If this is a truly random phenomenon this would occur only once in 4 000 000 pregnancies. Any tendency to form such lesions repeatedly should be possible to detect.

Similar placental lesions may be seen after induction of mid-trimester abortion with hypertonic saline (Christie *et al.*, 1966; Galen *et al.*, 1974). Another pointer to aetiology is that in two examples of Breus' mole in early abortions studied cytogenetically a 45 XO chromosome constitution was found (Wilkin, 1974, personal communication). In each instance a tiny blighted embryo was present.

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# RATE OF FALL IN PLASMA PROGESTERONE AND TIME TO ABORTION FOLLOWING INTRA-AMNIOTIC INJECTION OF PROSTAGLANDIN $F_{2\alpha}$, WITH OR WITHOUT UREA, IN THE SECOND TRIMESTER OF HUMAN PREGNANCY

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## Summary

Progesterone concentrations have been determined in a total of 175 peripheral plasma samples obtained serially from 20 women undergoing mid-trimester abortion. In ten patients abortion was induced by intra-amniotic administration of 50 mg. of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) alone; in ten others it was induced with 50 mg. of intra-amniotic $PGF_{2\alpha}$ with 80 g. of urea. Mean instillation-abortion intervals were 29·0 hours in the group receiving $PGF_{2\alpha}$ alone and 12·1 hours in the groups receiving $PGF_{2\alpha}$ and urea. In eight of the patients treated with $PGF_{2\alpha}$ and urea, and in four of those treated with $PGF_{2\alpha}$ alone, there were significant decreases in progesterone level (determined by calculation of correlation coefficients) during the instillation-abortion interval. When plotted against instillation-abortion times, calculated rates of decrease in peripheral plasma progesterone levels showed a statistically significant regression ($p<0·05$). This indicates that progesterone levels drop most rapidly in patients with shorter instillation-abortion times. There was no relationship between initial progesterone levels and instillation-abortion times.

INTRA-AMNIOTIC administration of $PGF_{2\alpha}$ or of other prostaglandins is widely used as a means of inducing mid-trimester abortion. Csapo (1973) has suggested that the mechanism by which intra-amniotic $PGF_{2\alpha}$ induces uterine contractility involves venoconstriction and contracture stimulated by exogenous prostaglandin, followed by continued cyclic activity maintained by prostaglandins of endogenous origin. It is accepted that rapid and complete abortion following intra-amniotic administration of $PGF_{2\alpha}$ is associated with decreasing peripheral plasma progesterone and oestrogen levels (Csapo *et al.*, 1972; Craft, Ferguson *et al.*, 1973; Tyack *et al.*, 1974). The results presented here suggest that there may be a relationship between the rate of decrease in progesterone levels and the instillation-abortion interval, the proges-

TABLE I
*Clinical details of patients*

Intra-amniotic PGF$_{2\alpha}$

| Patient No. | Age | Previous children | Gestation (weeks) |
|---|---|---|---|
| 1 | 31 | 2 | 13 |
| 2 | 22 | 4 | 16 |
| 3 | 29 | 1 | 16 |
| 4 | 40 | 10 | 14 |
| 5 | 27 | 5 | 15 |
| 6 | 18 | – | 16 |
| 7 | 33 | 2 | 14 |
| 8 | 17 | – | 20 |
| 9 | 27 . | 2 | 18 |
| 10 | 33 | 2 | 15 |
| Means (± S.E.M.) | 27·7±2·26 | 2·8±0·939 | 15 7±0·650 |

Intra-amniotic PGF$_{2\alpha}$ and 80 g. of urea

| Patient No. | Age | Previous children | Gestation (weeks) |
|---|---|---|---|
| 1 | 26 | 2 | 16 |
| 2 | 21 | 1 | 19 |
| 3 | 41 | 5 | 16 |
| 4 | 18 | – | 18 |
| 5 | 19 | 1 | 20 |
| 6 | 21 | – | 15 |
| 7 | 19 | – | 16 |
| 8 | 27 | 3 | 18 |
| 9 | 39 | 3 | 16 |
| 10 | 25 | 1 | 20 |
| Means (± S.E.M.) | 25·6±2·59 | 1·6±0·520 | 17·4±0·581 |

terone level dropping more rapidly in those patients aborting sooner.

## PATIENTS AND METHODS

Twenty obstetrically normal women undergoing legal termination of pregnancy were studied; each had given her informed consent to the removal of blood samples. Clinical details are given in Table I. After confirming the presence of a live fetus by ultrasound, and without premedication or local anaesthesia of the anterior adbominal wall, 50 mg. of PGF$_{2\alpha}$ either with (10 patients) or without (10 patients) 80 g. of urea was injected into the amniotic sac. Pulse, blood pressure and temperature were monitored at regular intervals. Antecubital venous blood, taken before (2 to 4 samples) and after (3 to 13 samples, at 3 to 5 hour intervals) the intra-amniotic injection, was heparinized and kept at 4 °C. until separation within 6 hours. Plasma was subsequently kept at −15 °C. until assay. Vaginal examinations were performed at 4-hourly intervals and the abortion time was taken as the presence of the conceptus in the vagina.

Progesterone was measured by radioimmunoassay in 0·1 ml. plasma samples after extraction with 3 ml. of petroleum ether by a method based on that of Morgan and Cooke (1972); this assay is described in detail elsewhere (Flint *et al.*, 1974).

## RESULTS

Instillation-abortion times for each patient are given in Table II; the mean value (±S.E.M.) in patients receiving PGF$_{2\alpha}$ alone was 29·0±

TABLE II

*Time between instillation of $PGF_{2\alpha}$, or $PGF_{2\alpha}$ and urea, and abortion, the rate of change of plasma progesterone and the correlation of progesterone level and time*

| Patient No. | Instillation—abortion time (hours) | Mean initial progesterone[1] level (ng./ml.) | Rate of change in progesterone (ng./ml./hr.) | Correlation coefficient (r) | p | Progesterone level in sample before abortion[2] (ng./ml.) |
|---|---|---|---|---|---|---|
| | | | $PGF_{2\alpha}$ alone | | | |
| 1 | 11 | 41·5 (3) | −2·55 (5) | 0 7606 | <0·10 | 7·5 |
| 2 | 45 5 | 20·0 (2) | −0·54 (6) | 0·8555 | <0·05 | 6·7 |
| 3 | 15·5 | 28·8 (2) | +0·07 (6) | 0 0411 | >0·10 | 30·9 |
| 4* | 88 | 65·3 (4) | +0·01 (4) | 0·0047 | >0·10 | N.D.[3] |
| 5 | 7·5 | 45·9 (2) | −1·86 (6) | 0·9564 | <0·001 | 10·8 |
| 6 | 17·5 | 31·9 (3) | −0 73 (8) | 0 8960 | <0·01 | 21·4 |
| 7 | 28 | 42·5 (2) | +0 54 (13) | 0·1980 | >0·10 | 42·7 |
| 8 | 48 | 46·1 (3) | +0·41 (10) | 0·6568 | <0·05 | 55·0 |
| 9 | 14 | 41·1 (3) | −1 50 (6) | 0·9274 | <0·01 | 22·3 |
| 10 | 15 | 41·3 (2) | +0 28 (4) | 0·1608 | >0·10 | 34·2 |
| | | | $PGF_{2\alpha}$ and urea | | | |
| 1 | 5·25 | 38·3 (2) | −4 01 (3) | 0·9999 | <0·01 | 18·3 |
| 2 | 15·5 | 32·2 (3) | −1·66 (6) | 0·7942 | <0·05 | 6·7 |
| 3 | 6·5 | 35·5 (2) | −2 02 (4) | 0·8804 | <0·05 | 17·6 |
| 4 | 8·5 | 50·9 (3) | −3·34 (8) | 0·8452 | <0·01 | 12·5 |
| 5 | 18 | 66·4 (3) | −2·86 (5) | 0·9700 | <0·01 | 10·0 |
| 6 | 11·5 | 29·4 (3) | −1·46 (7) | 0·7791 | <0·05 | 14·7 |
| 7 | 18 | 31 9 (4) | −1·41 (6) | 0·9199 | <0·01 | 8·3 |
| 8 | 6·5 | 62·9 (4) | −4·02 (3) | 0·9976 | <0·05 | 25·8 |
| 9 | 13·5 | 40·0 (3) | −2·62 (3) | 0·9835 | >0·10 | 22·9 |
| 10 | 18 | 46·1 (3) | −0·72 (6) | 0·5823 | >0·10 | 35·8 |

* Patient required oxytocin following spontaneous rupture of membranes.

[1] − indicates overall decreases; + indicates increases.

[2] value at sample taken preceding abortion; sampling time was up to 4 hours before abortion.

[3] last sample taken 53 hours before abortion, at administration of oxytocin.

(Values in parenthesis indicate number of plasma samples assayed.)

7·9 hours; after administration of $PGF_{2\alpha}$ with urea it was 12·1±1·6 hours ($0{\cdot}05<p<0{\cdot}10$). In the group treated with $PGF_{2\alpha}$ alone one patient subsequently required intravenous oxytocin infusion. Addition of urea to the abortifacient dose of $PGF_{2\alpha}$ resulted in a higher proportion of patients with falling progesterone levels (eight in the $PGF_{2\alpha}$ and urea group, compared with four in the group receiving $PGF_{2\alpha}$ alone), and also in more rapidly falling progesterone levels in those cases where a drop was observed (2·60±0·34 ng./ml. per hour in the $PGF_{2\alpha}$ and urea group compared with 1·44±0·37 ng./ml. per hour in the control group (Table II).

With the exception of two patients in the group receiving $PGF_{2\alpha}$ alone peripheral plasma progesterone levels at abortion were lower than before treatment; the mean value of the samples taken nearest to the time of abortion was 53·5±7·2 per cent of the mean of the pre-administration levels (Table II; for differences from 100 per cent $p<0{\cdot}001$). When progesterone levels were plotted against time following the intra-amniotic injection, statistically significant linear falling regressions ($p<0{\cdot}05$) were obtained for four patients receiving $PGF_{2\alpha}$ alone, and in eight patients treated with $PGF_{2\alpha}$ and urea (Table II).

In those cases with negative regression lines with significant correlation coefficients the rates of decrease in progesterone (i.e. the slopes of the lines obtained by plotting progesterone levels against time) were plotted against instillation-abortion times. The results show (Fig. 1) that

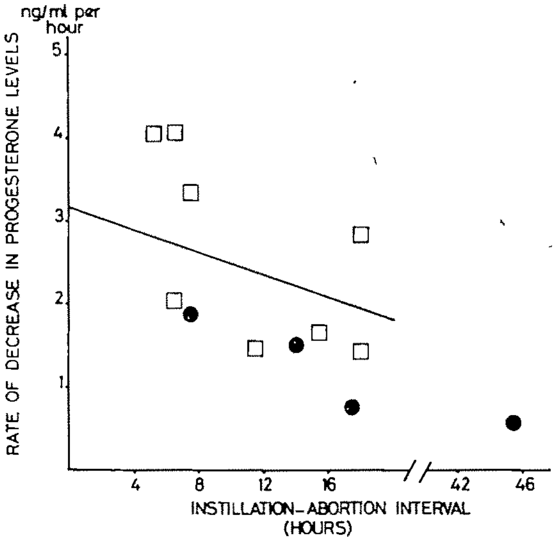

FIG. 1

Rates of decrease in progesterone level plotted against instillation—abortion time for patients showing significant negative regressions.

● $PGF_{2\alpha}$ □ $PGF_{2\alpha}$+Urea
$p<0{\cdot}05$ $r = 0{\cdot}6282$
$y = 0{\cdot}0679\ x = 3{\cdot}1034$

progesterone levels dropped most rapidly in the patients with shorter instillation—abortion times ($r = 0{\cdot}6282$; $n = 12$; $p<0{\cdot}05$). When mean pretreatment progesterone levels were plotted against instillation-abortion times, there was no significant regression ($p>0{\cdot}1$), indicating that patients with higher initial progesterone levels did not necessarily take longer to abort.

## DISCUSSION

The results of this investigation indicate that addition of urea to an intra-amniotic abortifacient dose of $PGF_{2\alpha}$ has three effects; it reduces the instillation-abortion time; it results in an increased proportion of patients who have decreasing progesterone levels; and it results in a faster decrease in progesterone level in those patients showing such a decrease. Intra-amniotic urea in combination with intra-amniotic prostaglandin has previously been shown to result in reduced instillation-abortion times without additional use of oxytocin (Craft, 1973; Craft *et al.*, 1973), and our results therefore confirm these findings. Our results also confirm the observations of Csapo *et al.* (1972), Craft *et al.* (1973) and Tyack *et al.* (1974) in that we have also shown that with higher doses of prostaglandins, and where instillation-abortion times are short, progesterone levels drop during the period immediately preceding abortion. In previous work where progesterone levels were shown to drop only in a minority of cases (Symonds *et al.*, 1972) relatively low doses of $PGF_{2\alpha}$ were used (25 mg.), and instillation-abortion times were longer (mean, 30 hours). In the patients described here, the mean instillation-abortion time in the five patients in whom no significant drop in progesterone level was observed, was 35·4±1·74 hours, significantly longer ($p<0{\cdot}025$) than in those where progesterone drop occurred (14·2±2·7 hours). The present results indicate that progesterone levels may not drop where instillation-abortion times are relatively long, suggesting that in these cases abortion was due to a direct effect of prostaglandins on the myometrium.

There appears to be no consistent causative inter-relationship between uterine contractions and decreasing progesterone levels. Uterine activity may occur in the absence of any drop in progesterone level, as seen in patients receiving $PGF_{2\alpha}$ alone (Table II; see also Symonds *et al.*, 1972); therefore it seems likely that increased uterine activity results from decreased progesterone levels, or that decreasing progesterone levels result from uterine activity. Separate explanations must be sought for these two effects.

Uterine contractility reflects myometrial activity, and since progesterone is a product of the placenta, decreased progesterone levels probably reflect damage to the placenta. There may therefore be an *a priori* case for considering these effects separately. Csapo (1973) has reviewed the evidence for a direct effect of $PGF_{2\alpha}$ on the myometrium, and has suggested that the initial contracture it causes may result in vasoconstriction, and hence in a transient placental ischaemia; this may be the cause of the decreasing progesterone levels observed in some patients receiving $PGF_{2\alpha}$ alone. A direct effect of $PGF_{2\alpha}$ on the myometrium is consistent with the stimulation of cyclic uterine activity (Symonds *et al.*, (1972).

Effects of osmotic damage to the placenta, by intra-amniotic administration of hypertonic solutions of saline, glucose and urea, are well documented (Turnbull and Anderson, 1965; Christie *et al.*, 1966; Gustavii and Green, 1972), and include effects on maternal progesterone levels (Csapo *et al.*, 1969; Holmdahl *et al.*, 1971; Tyack *et al.*, 1973). The dropping levels of progesterone seen more frequently in patients receiving PGF$_{2\alpha}$ and urea may well result, therefore, from such an osmotic effect.

The possibility of separable effects of intra-amniotic PGF$_{2\alpha}$ and urea may well explain the occurrence of abortion without dropping progesterone levels, as seen previously (Symonds *et al.*, 1972) and, in this investigation, following administration of PGF$_{2\alpha}$ alone. However, it does not explain the shortening of the instillation-abortion time, by the addition of urea to the abortifacient. This may occur by virtue of the removal of the well-known uterine-inhibitory action of progesterone, as the levels drop. The removal of the progesterone block has been implicated in the mechanism of the spontaneous onset of labour at term (Csapo, 1961), and falling progesterone levels have been observed at term (Csapo *et al.*, 1971; Turnbull *et al.*, 1974). Inasmuch as the present investigation has shown progesterone levels drop faster in patients aborting sooner it supports the suggestion that removal of the progesterone block facilitates labour, although whether this is due to an effect on the myometrium, the cervix, or both, is as yet not known.

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# EFFECT OF PROTEIN HORMONES AND STEROIDS ON TISSUE CULTURES OF ENDOMETRIAL CARCINOMA

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## Summary

Neoplastic tissue was obtained from 30 patients with endometrial carcinoma and the response of this tissue to various hormones was studied *in vitro* by histological methods and by measuring the incorporation of $^3$H thymidine into DNA. Progesterone and synthetic progestogens had a consistently adverse effect on tissue survival *in vitro* while pregnenolone had a beneficial effect.

IT has been suggested that there may be an association between endometrial cancer and oestrogens (Greene, 1957; Frachtman, 1961; Dallenbach-Hellweg, 1964; Gusberg, 1967; Gusberg and Kardon, 1972). Endometrial cancer occurs in more than 60 per cent of cases in postmenopausal women who have clinical evidence of persistent oestrogen production (Hustin, 1973*a*).

It has also been found that treatment with progesterone or progestogens can give substantial remissions in more than one-third of patients with advanced cancer of the endometrium (Anderson, 1965; Kistner *et al.*, 1965; Kennedy, 1968) and histological studies show an arrest of proliferation and a drop in the mitotic index. Simultaneously, glycogen and mucosubstances accumulate within the cells and in the gland lumina (Hustin, 1973*b*). But the biochemical action of progesterone on the cancer cell is still uncertain (Trams *et al.*, 1973). Taylor (1973) could not find a modification of cytoplasmic binding sites for oestradiol in two patients with endometrial cancer treated by progestogens. Nordqvist (1964, 1970 and 1973), however, showed a decrease of DNA synthesis after two hours incubation of endometrial cancer tissue with progesterone.

The purpose of this paper is to describe the action *in vitro* of several steroids or peptide hormones on endometrial cancer tissue in terms of DNA synthesis and survival of tissue cultures.

## METHODS

### *Specimens*

*Cancers.* Thirty postmenopausal patients with endometrial carcinoma were studied; 24 of the tumours were well differentiated and 6 were undifferentiated or anaplastic. Sixteen specimens were obtained by curettage, without previous treatment. Nine patients had already had one or two weekly doses of 1 g. of medroxyprogesterone acetate and five patients had additionally been treated with intra-uterine radium.

*Controls.* Eight specimens of normal endometrium were obtained between days 8 and 10 (proliferative phase) of the cycle and nine specimens between days 22 and 25 (luteal phase). There were also four specimens of hyperplastic endometrium obtained during prolonged anovulatory amenorrhoea. Small fragments of myometrium were obtained from three patients having hysterectomy for benign disease.

Biopsies obtained from a moderately well-differentiated epidermoid carcinoma arising in the main bronchus of a 65-year-old man and from a lymphosarcoma arising in the nasal sinuses of a 50-year-old woman were also studied.

*Experiments*

In vitro *survival procedures*. In each experiment, 1 cu. mm. fragments of tissue were cut under aseptic conditions and thoroughly washed with Parker-199 (Difco) containing penicillin (600 U./ml.). Two fragments were picked out simultaneously, and placed on stainless steel grids, siliconed paper squares or "Gelfoam" cubes in a 35 mm. diameter Petri dish. The basic culture medium contained Parker TC 199 (Difco) supplemented with 10 per cent adult blood donor serum, crystalline insulin (0·1 unit/ml.), glucose (2 mg./ml.) and penicillin (400 units/ml.).

Steroid hormones were dissolved in ethanol-saline solutions which were diluted to a final concentration of 0·01 per cent ethanol/ml. of medium; untreated cultures were supplemented with the same amount of ethanol-saline solution without hormones. The final concentrations of steroids in the medium were: oestradiol-17-β (10 to 20 μg./ml.), oestriol (10 to 10 μg./ml.), pregnenolone (25 μg./ml.), progesterone (60, 80 and 100 μg./ml.), 17α-hydroxyprogesterone (60 μg./ml.), 17α-hydroxyprogesterone-19-nor-capronate (100 μg./ml.), norethisterone acetate (20 μg./ml.), and ethylestrenol (80 μg.△ml.). In some experiments peptide hormones dissolved in saline were also added with or without steroids and in the following amounts: growth hormone (2 units/ml.) and human chorionic gonadotrophin (100 IU./ml.).

The culture vessels were then incubated at 37 °C. in a moist chamber and for 30 minutes every day were treated with 95 per cent oxygen and 5 per cent $CO_2$. The medium was changed only once at three days. At various intervals up to seven days, tissue fragments were picked out, washed in saline and fixed in Bouin's solution. After embedding in paraplast, they were serially cut at 4 μ. and stained either by haemalun,

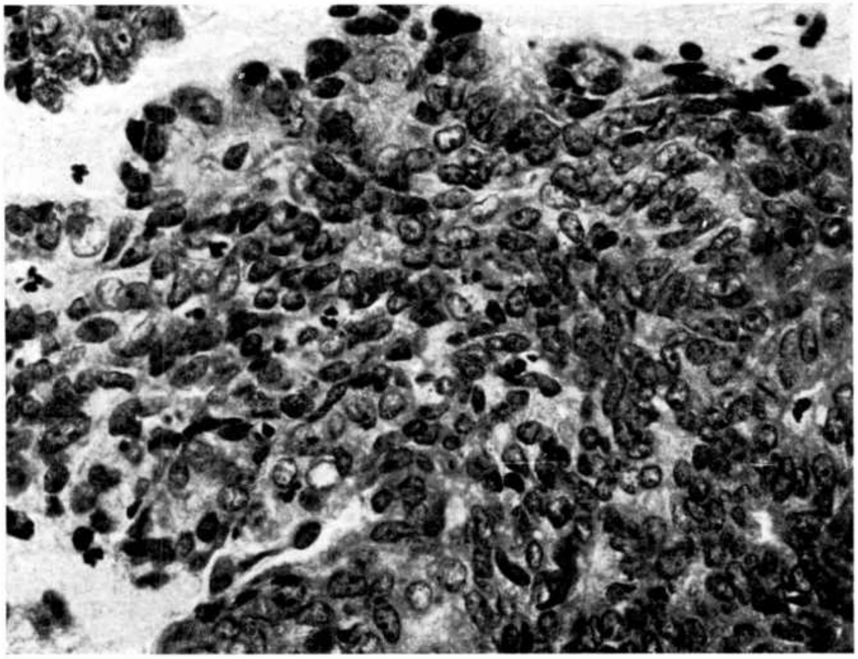

FIG. 1

Good survival of tissue culture of endometrial carcinoma at seven days. No hormones were added. (Haemalun, erythrosine and saffran × 120).

erythrosine, saffran, or by PAS for assessment of neutral and acidic mucosubstances. These sections were compared with previously prepared diagnostic slides. Survival was assessed according to an arbitrary scale, and divided into three grades. *Good survival* meant an absence of any sign of necrosis (Fig. 1). In the most favourable cases, evidence of proliferation with mitoses and penetration of isolated cells into fibrin strands in the case of the "Gelfoam" technique could be seen. *Moderate survival* implied the presence of patchy necrosis. *Poor survival* implied extensive necrosis or no survival at all.

*Autoradiographic studies.* One cu. mm. fragments of endometrial cancer were incubated with $^3$H-thymidine (final concentration : 4 $\mu$Ci./ml.) in TC 199. Oestradiol, progesterone or pregnenolone were added to the incubation medium together with the labelled precursor. Their concentrations were respectively 5, 10 and 10 $\mu$g./ml. of TC 199. In some experiments, tumour fragments were incubated with oestradiol-17β, progesterone or pregnenolone for 60 to 120 minutes prior to adding the DNA precursor. Finally, and two hours before thorough washing in saline, fixation in Bouin's solution and embedding in paraplast, tritiated thymidine (4 $\mu$Ci./ml.) was added to 3- or 7-day-old cultures. Blocks were cut serially at 4 $\mu$. The slides were deparaffinized, brought to water and dried. They were then coated, in the dark with Ilford liquid autoradiographic emulsion, redried and kept in light proof boxes at 4 °C. for 15 to 30 days, After development and fixation, the slides were counterstained with Harris haematoxylin. The $^3$H-Index was estimated by establishing the ratio of:

$$\frac{\text{marked cells}}{\text{total cells}} \times 100, \text{ in a count of 400 cells.}$$

Three or four slides per culture were usually screened.

## RESULTS

### *Non-neoplastic endometrium*

After seven days culture, tissue survival was considered good or very good (Grade 1) in 17 instances (81 per cent) (Table I). Addition of oestradiol-17β did not influence the figures. On the other hand, progesterone was associated with a low survival rate and frequent necrosis of proliferative or hyperplastic endometria. Though the number of cases was limited, progesterone did not seem to influence the behaviour of secretory endometrium (Table II). Strips of myometrium survived well without necrosis and the picture was not influenced by the addition of hormones.

### *Endometrial cancer*

*Basal conditions.* After seven days culture, 12 samples (40 per cent) showed good to very good survival (Grade 1), while eight specimens (26 per cent) looked viable with areas of patchy necrosis (Grade 2). The other ten cultures showed Grade 3 changes with extensive or complete necrosis (Table III). Owing to frequent infection of the culture, tissue from previously irradiated cancers usually did not grow well. Anaplastic cancers were always scored Grade 2 or 3. Cultures made from tissue with a mitotic index under 12 per thousand seemed to grow better than cultures with a higher mitotic index (Fig. 2), but the difference had no statistical significance.

*Protein hormones.* Growth hormone and HCG did not alter the survival of cells in tissue culture nor did they modify the influence of steroid hormones simultaneously added to the cultures.

*Steroid hormones.* There was a slight but

TABLE I

*Non-cancerous endometrium.* In vitro *survival after seven days*

| Appearance of cultures after seven days | Functional status of the endometrium | | | |
|---|---|---|---|---|
| | Proliferative | Secretory | Hyperplastic | Total |
| Good survival | 7 | 7 | 3 | 17 |
| Moderate survival | 1 | 1 | – | 2 |
| Poor survival | – | 1 | 1 | 2 |
| Total | 8 | 9 | 4 | 21 |

TABLE II

*Non-cancerous endometrium.*
*Effects of steroid administration on tissue cultures*

| Appearance of cultures after seven days | | Functional status of the endometrium | | |
|---|---|---|---|---|
| | | Proliferative | Secretory | Hyperplastic |
| + oestradiol-17$\beta$ | Good survival | 3 | 2 | 1 |
| | Moderate survival | – | – | 1 |
| | Poor survival | 1 | 1 | 1 |
| + progesterone | Good survival | 1 | 2 | 1 |
| | Moderate survival | – | – | – |
| | Poor survival | 3 | 1 | 2 |

TABLE III

*Endometrial cancer.* In vitro *survival after seven days according to the preliminary status of the tumour*

| Appearance of cultures after seven days | Untreated cancers | Cancers treated with progestogens | Cancer irradiated and treated with progestogens | Total |
|---|---|---|---|---|
| Good survival | 7 | 3 | 2 | 12 |
| Moderate survival | 3 | 3 | 2 | 8 |
| Poor survival | 6 | 3 | 1 | 10 |
| Total | 16 | 9 | 5 | 30 |

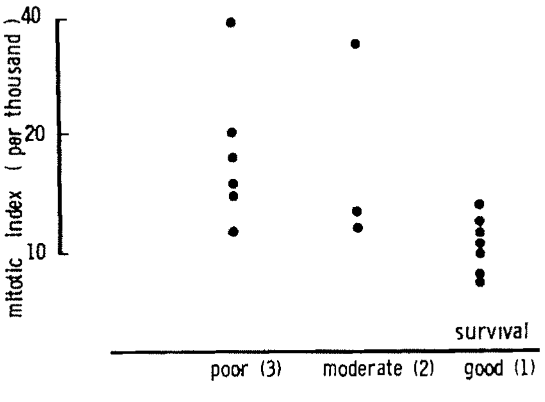

FIG. 2
Survival of endometrial cancer tissue cultures according to mitotic index of tumour.

insignificant decrease of tissue culture survival when oestradiol-17β was added. When progesterone was added, there was a sharp increase in the amount of necrosis (Table IV) and no culture showed good survival of cells. The effect was most marked in tissue obtained from patients treated with hormones or by irradiation (Table V). Derivatives of 17α-hydroxyprogesterone or of 19-nor-testosterone also induced in all cases a considerable loss of viability of the tumour culture. In contrast, pregnenolone added to tissue from eight untreated patients enhanced the viability of tumour cultures while progesterone induced extensive necrosis (Grade 3 changes) in seven instances (Table VI).

Finally, the two non-hormone dependent

TABLE IV

*Survival of endometrial cancer tissue culture according to steroid hormone added*

| Appearance of tissue culture after seven days | No addition to medium | Addition of oestradiol-17β to medium | Addition of progesterone to medium |
|---|---|---|---|
| Good survival | 12 | 9 | – |
| Moderate survival | 8 | 7 | 11 |
| Poor survival | 10 | 14 | 19 |

TABLE V

*Survival of endometrial cancer tissue culture according to previous treatment and to steroid hormone added*

| Treatment of patient before tissue was obtained for culture | Appearance of tissue culture | No addition to medium | Addition of oestradiol-17β to medium | Addition of progesterone to medium |
|---|---|---|---|---|
| None | Good survival | 7 | 5 | – |
| | Moderate survival | 3 | 4 | 7 |
| | Poor survival | 6 | 7 | 9 |
| Progestogens | Good survival | 3 | 4 | – |
| | Moderate survival | 3 | 1 | 3 |
| | Poor survival | 3 | 4 | 6 |
| Progestogens and irradiation | Good survival | 2 | – | – |
| | Moderate survival | 2 | 2 | 1 |
| | Poor survival | 1 | 3 | 4 |

cancers survived well in tissue culture irrespective of the steroid hormone added.

*$^3$H-thymidine incorporation.* Cancer tissue from untreated patients which was incubated with $^3$H-thymidine, had a mean labelling index of 22 per cent. Incubation with oestradiol-17β for 120 minutes did not alter the ability of cells to incorporate the DNA precursor. Progesterone caused a slight decrease of cell-labelling but it was not significant (Fig. 3). Previous treatment of cancer with progestogen caused a significantly lower labelling index (mean value = 4 per cent) when compared with cultures made from untreated patients ($p<0 \cdot 01$). The labelling index remained low when oestradiol, pregnenolone or progesterone were added to the incubation medium simultaneously with thymidine. When $^3$H-thymidine was added after a preliminary incubation of two hours, the labelling index was still 95 per cent of the initial values in basal conditions. When the medium had been initially supplemented with oestradiol-17β the decrease of DNA synthesis was no greater than in basal conditions. Preliminary contact with progesterone induced a slight but insignificant decrease of the $^3$H-index (80 per cent of the initial value). After four hours, the percentage of labelled cells in contact with progesterone fell to 50 per cent of initial values, while with oestradiol or in hormone-free medium, the values found were

TABLE VI

*Survival of endometrial carcinoma tissue in different hormonal environments*

| Appearance of cultures after seven days | No addition to medium | Addition of oestradiol-17$\beta$ to medium | Addition of progesterone to medium | Addition of pregnenolone to medium |
|---|---|---|---|---|
| Good survival | 4 | 2 | – | 8 |
| Moderate survival | 1 | 1 | 1 | – |
| Poor survival | 3 | 5 | 7 | – |

still 80 per cent (Fig. 4). Pregnenolone, on the other hand, did not change the labelling index in two or four hours.

After three days culture, the $^{3}$H-index fell in all experiments. With progesterone and oestradiol-17β it was no longer measurable. In balas conditions, it remained at 20 per cent, while with pregnenolone, the $^{3}$H-index was still 60 per cent of the initial value. At seven days, labelled cells were found only in cultures made in the presence of pregnenolone (40 per cent of initial value).

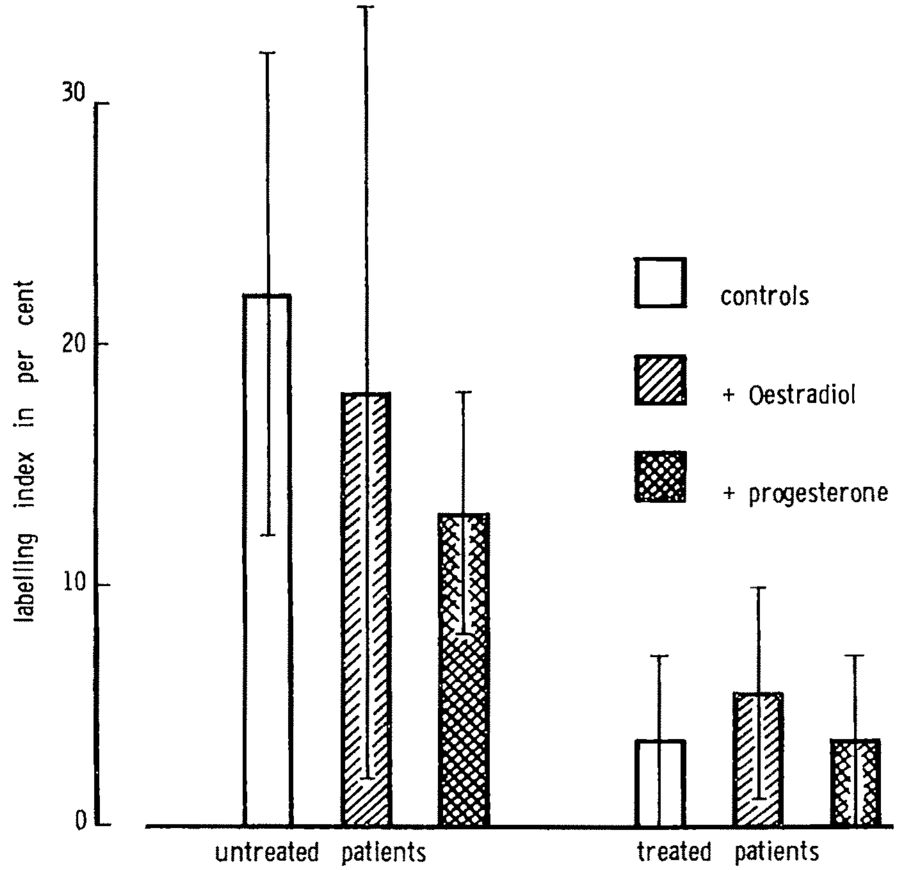

FIG. 3

Tritiated thymidine incorporation in endometrial cancer from patients who were untreated or treated by progestogens (mean values ±2 standard deviations). There is a marked decrease of the $^{3}$H-index in treated cases. Addition of hormones to the incubation did not alter the labelling indices.

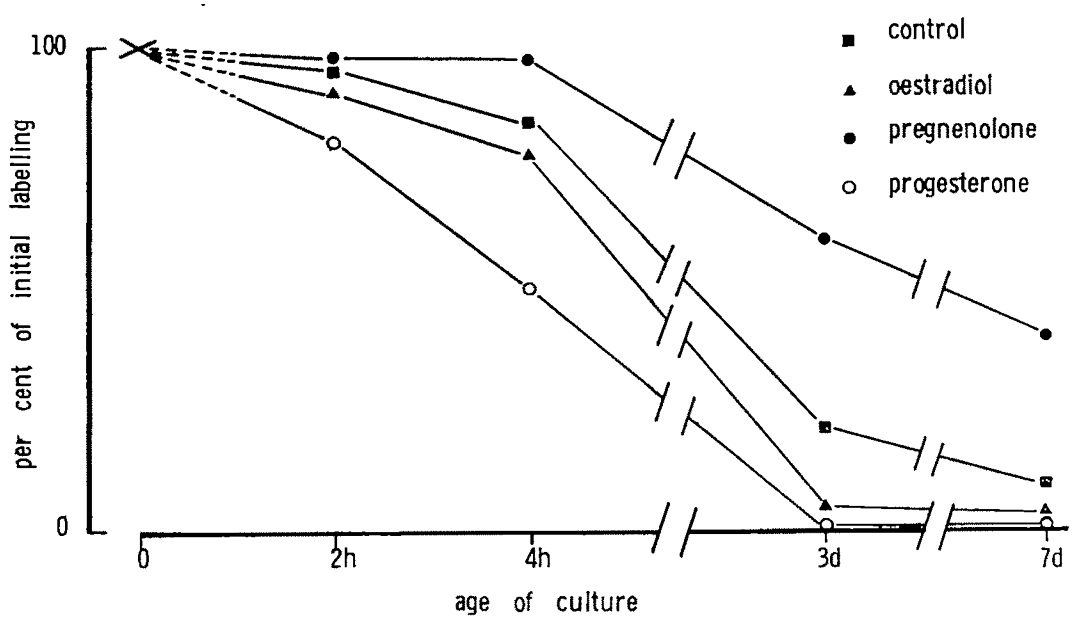

FIG. 4
Variations of the $^{3}$H-thymidine incorporation according to the duration of exposure to steroid hormones.

### COMMENTS

Cultures of endometrial carcinoma tissue seemed to survive well if the medium was supplemented with proteins, glucose and insulin. The last hormone was necessary to reach a 50 per cent survival rate in basal conditions. Protein hormones such as growth hormone or HCG did not influence the behaviour of endometrial cancer tissue cultures, while progesterone impaired their survival and caused, after four hours contact, a significant reduction of $^{3}$H-thymidine incorporation which might explain the low mitotic index frequently encountered in endometrial cancers treated by progesterone or progestogens (Hustin, 1973*a*). If, after seven days of culture, viable tissue could be found, it showed a secretory pattern.

The high dosage of progesterone used in tissue cultures could, theoretically, have a non-specific inhibitory effect. However, Nordqvist (1973 and unpublished data) has shown that in the culture system used, progesterone is, in large part, bound to serum proteins present in the medium (most probably corticosteroid-binding globulin). The 50 per cent inhibition of DNA synthesis, after four hours contact with progesterone is most interesting. After two to four hours incubation with progesterone, only those cells which are very close to the S-phase (pre-mitotic DNA synthesis phase) can take up tritiated thymidine. Das and Martin (1973) showed, in the mouse, that progesterone blocked endometrial cells in the early $G_1$ resting pre-synthesis phase ($G_1$) and that it was without effect upon their passage through late $G_1$, (resting post-synthesis), S and pre-mitotic phase ($G_2$). We could also postulate that progesterone blocks cancer cells in early $G_1$ and thus diminishes the number of cells able to take up the DNA precursor. This is then followed by secretory conversion (rarely demonstrated) in the experiments described here) and finally by cell death and necrosis. This sequence was not seen in cultures made from myometrium or non-hormone dependent cancers, and only to some extent with normal secretory endometrium. Endometrial cancer behaves like proliferative or hyperplastic endometrium and Brush (1973) has commented on the biological and endocrinological similarities between endometrial cancer and normal uterine endometrium. Indeed, most endometrial cancers seem to retain steroid-binding sites for oestradiol and for progesterone (Edwards, 1973; Taylor, 1973), both of which are hormones which control nucleic acid metabolism (Leroy, 1974).

The rather extraordinary enhancement of tissue culture caused by pregnenolone is difficult to explain because pregnenolone is a precursor of progesterone, being involved in the first step of the $\triangle_5$ pathway in C19 steroid and oestrogen biosynthesis. One might therefore expect that pregnenolone would be peripherally metabolized to progesterone and that it would thus have the same effect as progesterone on tissue cultures, albeit with some delay. But this is obviously not the case. Endometrial cancers may therefore have an unknown steroid metabolic pathway in which pregnenolone plays an important role even after seven days of tissue culture.

## ACKNOWLEDGEMENT

The invaluable help of Dr. J. M. Binard in providing the tissue for our experiments is gratefully acknowledged.

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# AN OPERATION FOR RESTORATION OF URINARY CONTINENCE FOLLOWING TOTAL LOSS OF THE URETHRA

BY

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## Summary

Twenty-three patients with urethral loss have been treated by the simple creation of a "spout" from the bladder wall. The procedure is suggested for the surgical treatment of lymphopathia venereum and for consideration in certain other conditions when urinary diversion is unacceptable.

THERE have been various theories concerning the mechanism of urinary continence in the female (Cameron, 1964; Lapides *et al.*, 1960; Jeffcoate and Roberts, 1952; Hutch, 1967). Most of them ascribe a major or minor role to the urethra including its angular relationship to the bladder, although the urethra does not normally contain urine except during the act of micturition. There is little disagreement that the urethra does provide an important secondary defence mechanism against involuntary leakage, but most gynaecologists would also believe that a patient deprived of her entire urethra would be hopelessly incontinent without a major reconstruction operation (Howkins, 1968).

When reconstructive operations have been deemed inappropriate, it has been usual to advise urinary diversion, but in developing countries this solution is unpopular because an abdominal stoma is unacceptable. Even if this were not the case, suitable bags and other apparatus tend to perish in the tropics. Ureterocolic anastomosis is a procedure which has obvious defects and itself depends on integrity of the rectum and anal sphincter mechanism (Hudson, 1970).

We have been concerned with attempts to restore, without performing a major plastic procedure, an acceptable degree of continence to the few unfortunate women who suffer total loss of the urethra.

## PATHOLOGY

There are three main causes for total loss of the urethra. The first is lymphopathia venereum, an inflammatory and granulomatous process which involves the lower genital tract and anorectal region (Douglas, 1962). It commonly produces an anorectal stricture and rectovaginal fistula which contraindicate ureterocolic anastomosis. Anteriorly there is total destruction of the urethra with the formation of a suburethral hood which is the remnant of the anterior vaginal wall. Lymphopathia involves considerable fibrosis with gross impairment of the local blood supply which dooms to failure both plastic operations for urethral reconstruction involving flaps or pedicles, and attempts to close the fistulae (Lawson, 1963).

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Based on a paper presented at 20th British Obstetric and Gynaecological Congress.

The next group of patients are those with malignant disease of the vulva and external meatus, the so-called vulvo-urethral type of carcinoma of the female urethra (Riches and Cullen, 1951). Carcinoma of the urethra seems to be slightly more common in negro than in white races. If the tumour encroaches on to the lower vagina a radical operation must remove at least half of the vagina and the full length of the urethra, leaving a hole of variable size in the bladder base. Such an operation is an extension of the standard radical vulvectomy but stops short of anterior exenteration which, in some circumstances, may be appropriate (Hudson, 1966).

Thirdly, urethral loss may be due to trauma. Total urethral ablation has been produced by ritual female circumcision which is still performed in some countries. Total urethral destruction is only rarely a sequel to obstructed labour, which usually damages only the upper urethra and bladder base.

## OPERATIVE TECHNIQUE

The extent of the surgical problem is illustrated by the artist's impression which shows the relationship of the truncated bladder to the arch of the pubis (Fig. 1). The surgical procedure we have adopted is the simple construction of a "spout" by plicating the side and inferior part of this gaping hole in the bladder base, with "reefing" sutures (Fig. 2). Extra-chromic catgut No. 2/0 has been used but polyglycollic acid would be suitable.

When the procedure has been carried out for lymphopathia venereum, we have used a semicircular incision into the suburethral hood which has given access to the inferior aspect of the bladder into which these sutures have been placed. In this instance there has usually been no need to fix the new bladder neck to the back of the symphysis pubis but in the traumatic cases and, in particular, those associated with

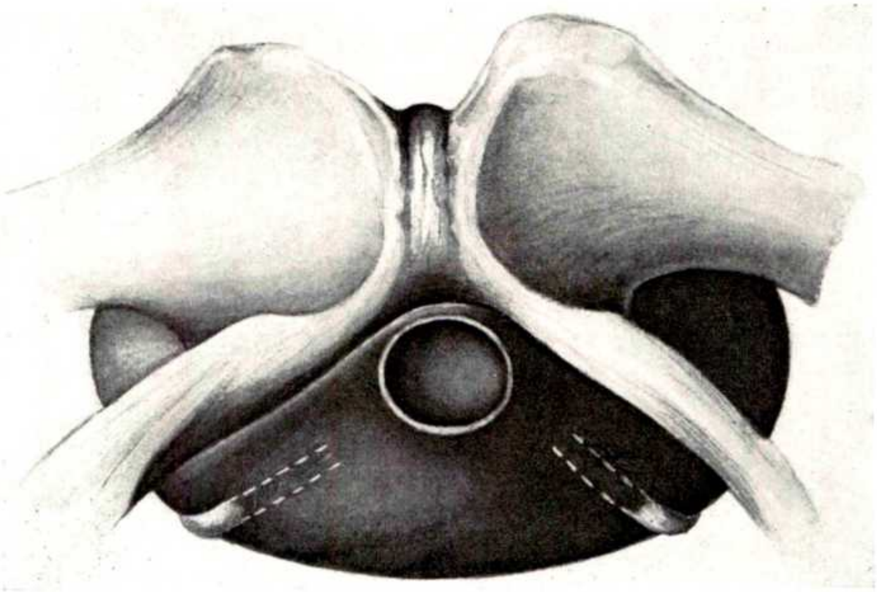

FIG. 1

Drawing of the truncated bladder base above and behind the symphysis pubis seen from below.

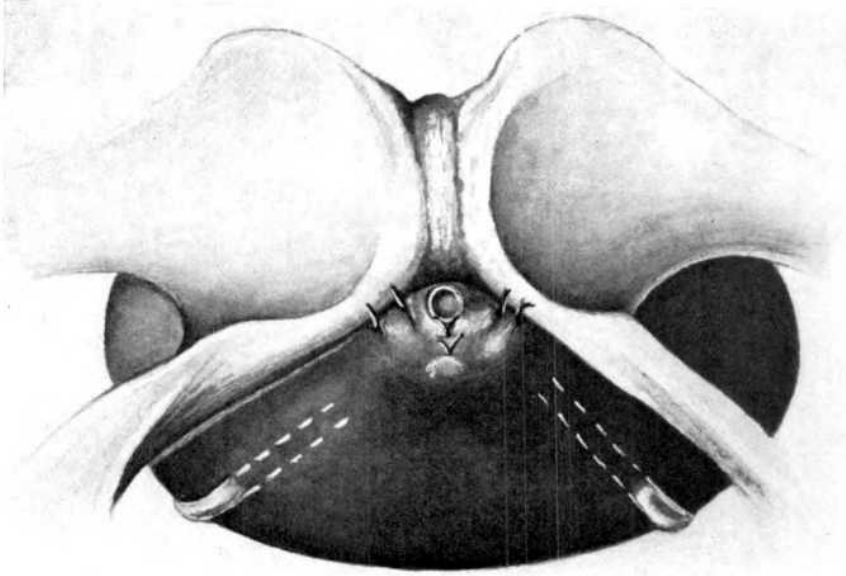

FIG. 2

Drawing, seen from below, of the "spout" created by inserting "reefing" sutures around the hole in the bladder base. When necessary this spout is advanced and fixed by further sutures to the interior part of the arch.

the surgical ablation of carcinoma, some further sutures are required. In these surgical cases the situation is a little more complicated because not only has the urethra been removed but the lower half of the vagina is missing as well. The normal position of the bladder is well above the superior surface of the pubic bones and any discharge of urine in this position inevitably flows across the back of the symphysis pubis. In these cases it has been necessary not only to construct an urethral spout, but also to advance both the new bladder neck and the vagina to the inferior aspect of the symphysis pubis. If this advancement is not achieved, urine collects in the cave of Retzius causing osteitis pubis which may be very painful, We have, therefore, combined plication of the hole in the bladder base with mobilization of the upper stump of the vagina so that it can be sutured to the periosteum under the arch of the pubis, bringing the new urethral "spout" down with it.

The results of this relatively simple operative procedure are shown in Table I which also gives the incidence of the various aetiologies. Figure 3 is a still X-ray from a micturating cystogram performed on a patient who regained continence after operation.

As an alternative to ureterocolic anastomosis or the creation of an unacceptable urinary conduit the degree of continence achieved by this measure has been extremely welcome. This relatively simple procedure merits a trial before more drastic measures are undertaken.

TABLE I

*Aetiology of total loss of urethra and results of 23 operations to restore continence*

| | |
|---|---|
| *Pathology* | |
| Carcinoma (vulvo-urethral) | 8 |
| Lymphopathia venereum | 9 |
| Trauma (miscellaneous) | 6 |
| *Follow-up* | |
| Three months to five years | |
| *Result* | |
| Good (continent) | 17 |
| Fair (moderate stress) | 2 |
| Poor (severe stress) | 4 |
| Total failure | 0 |

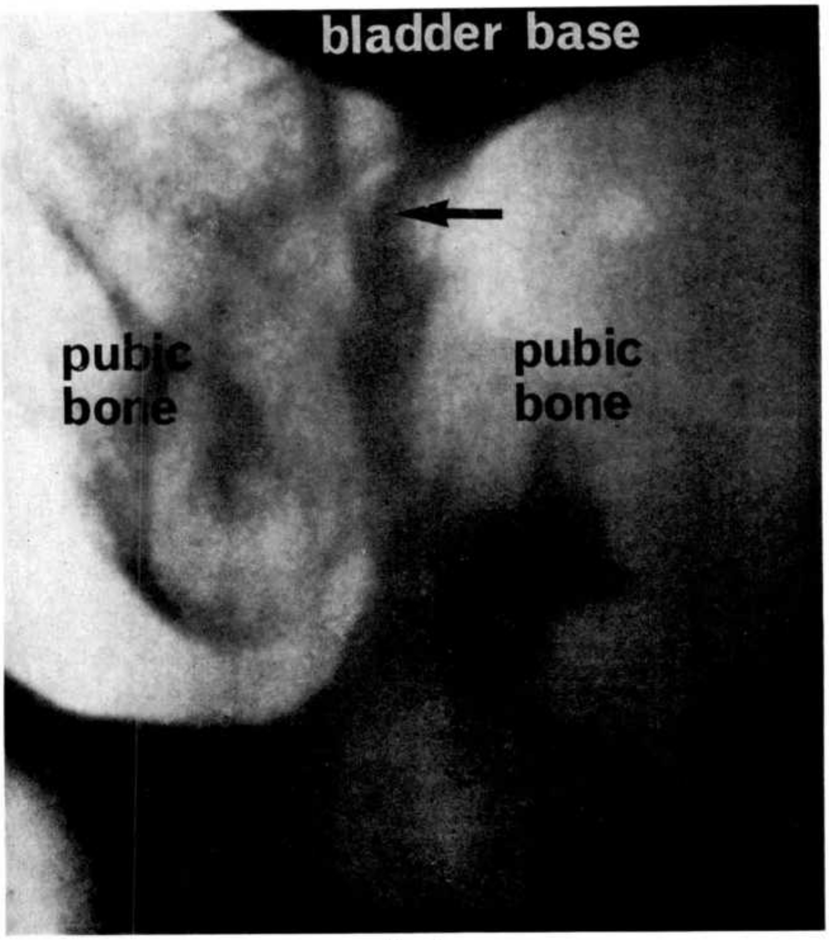

FIG. 3

Cystogram showing the new "bladder neck" during voluntary micturition.

### ACKNOWLEDGEMENTS

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# MYOCARDIAL INFARCTION AND RHEUMATIC HEART DISEASE IN PREGNANCY
## CASE REPORT

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### Summary

Myocardial infarction is a rare complication of pregnancy, only 45 proven cases having been reported. In only one patient was rheumatic heart disease an associated factor (Goldberger and Pokress, 1950).

### CASE REPORT

A WOMAN, aged 43 years, gravida 4, para 3, was admitted to hospital as an emergency at 26 weeks gestation in her fourth pregnancy with severe retrosternal chest pain of sudden onset accompanied by breathlessness and sweating.

She had had rheumatic fever at the age of 13 years and was known to have rheumatic heart disease. During the antenatal period prior to her admission she had been examined at fortnightly intervals by a physician who had detected no evidence of cardiac decompensation. Apart from pre-eclampsia during her first pregnancy at the age of 25 years, she had remained well throughout her previous confinements with no episodes of cardiac failure.

On examination, she had "mitral facies" and was slightly dyspnoeic at rest. Her blood pressure was 130/80 mm. Hg. and her pulse 80 beats per minute in sinus rhythm. Crepitations were present at both lung bases. Examination of her heart revealed cardiac enlargement, the apex beat being displaced laterally to the anterior axillary line, and murmurs commensurate with a diagnosis of mitral stenosis, mitral incompetence and aortic stenosis. An electrocardiogram showed ST depression in chest leads $V_3$-$V_6$ indicating an inferior myocardial infarction. Her chest X-ray confirmed cardiomegally and engorgement of the pulmonary vessels consistent with advanced rheumatic heart disease. The serum asparate aminotransferase (SGOT) was elevated to 108 IU/l; serum lactic dehydrogenase (LDH) was elevated to 680 units/ml and the serum alanine aminotransferase was 8 IU/l which was within the normal range.

Initially, she was treated with 10 mg. of diamorphine intramuscularly and with 4-hourly intravenous injections of 5000 IU of heparin for 12 days after which time anticoagulant therapy was reduced gradually over a 48-hour period, stopped and the patient then mobilized. She was discharged from hospital at 30 weeks gestation after a total of one month in-patient care.

Thereafter she attended the antenatal clinic at weekly intervals. She remained normotensive; her haemoglobin was in excess of 11·0 g/100 ml. and there was no evidence of cardiac decompensation.

At 266 days gestation she was readmitted into hospital for rest and went into spontaneous labour at 279 days gestation. Intramuscular ampicillin, 500 mgs. 6 hourly, was commenced. The first stage of labour lasted 2 hours 25 minutes during which time she received a single intramuscular injection of 15 mg. of morphine intramuscularly for analgesia. A prophylactic forceps delivery was performed and a healthy female infant with an Apgar score of 10 at one minute was delivered. The baby weighed 3·095 kg.

Immediately after delivery, the patient became dyspnoeic and cyanosed and crepitations were audible at both lung bases. She experienced no chest pain. Oxygen was given via a face mask and 40 mg. of frusemide was administered intravenously. Oxytocics were initially withheld but a sudden postpartum haemorrhage of 1000 ml. from a uterus which remained atonic despite bimanual massage, necessitated the intravenous administration of 0·25 mg. of ergometrine. The vaginal haemorrhage ceased and her cardiovascular status rapidly improved. An electrocardiograph at this time revealed no evidence of fresh infarction.

On the first day after delivery she was mobilized and treatment with 40 mg. of oral frusemide daily and potassium supplements commenced. Oral ampicillin, 500 mg., was given six-hourly for the first five postpartum days. Anticoagulant therapy was considered unnecessary and oestrogens were withheld for suppressing lactation, mild analgesics and firm breast binding being used to overcome mild engorgement. The haemoglobin concentration 72 hours following delivery was 10·8 g/100 ml. The patient remained well throughout the puerperium and was discharged on the fourteenth day postpartum on potassium supplements, diuretic and haematinic therapy.

Postnatal examination at 6 weeks showed no evidence of cardiorespiratory embarrassment and the diuretic and potassium supplements were discontinued.

Because of the risk of a similar thrombotic episode in a future pregnancy, sterilization was advised. However her husband had elected to have a vasectomy operation performed in the near future.

## DISCUSSION

Reviewing myocardial infarction during pregnancy, Husaini (1971) shows that in only one previous case was rheumatic heart disease a complicating factor (Goldberger and Pokress, 1950). In this particular case, the patient, aged 37 was also known to have tertiary syphilis.

Diagnosis of myocardial infarction in the pregnant patient presents clinical difficulties. Fletcher *et al.* (1967) and Ginz (1970) state that the most reliable evidence of obstructive coronary disease depends on electrocardiographic analysis after allowing for normal pregnancy changes.

Management of myocardial infarction in pregnancy involves treatment of the acute episode and attention to the safest method of management for the remainder of the pregnancy.

Initial therapy does not differ from that used in non-pregnant patients with the exception that anticoagulants are used more liberally. Thirteen of the 45 previously described patients with myocardial infarction in pregnancy received anticoagulants. Hirsh *et al.* (1970) state that choice of anticoagulant and careful control are important and its maintenance after mobilization of the patient of questionable value, a policy which was adopted in this patient. Should angina persist following treatment, termination of the pregnancy would appear to be of therapeutic value (Pfaffenschlager, 1964; Listo and Bjorkenheim, 1966; Husaini, 1971).

The management of labour poses certain difficulties. Spontaneous onset of labour should be anticipated, but if for other reasons induction of labour is indicated it is suggested that a technique of low amniotomy with simultaneous intravenous infusion of prostaglandin be adopted under antibiotic cover. The antidiuretic effect of oxytocin at term (Abdul-Karim and Assali, 1961; Assali *et al.*, 1960; Douglas, 1965) and intrinsic ability of oxytocin to affect myocardial function and coronary flow (Pauerstein, 1973) contraindicate its use for the induction of labour. Prostaglandins have no antidiuretic properties or detrimental effects upon the cardiovascular system of pregnant women (Karim *et al.*, 1971). Indeed, animal experiments have shown that prostaglandins, particularly the E series have pronounced coronary vasodilator action (Mantegazza, 1965; Vergraesen *et al.*, 1967 and Willebrands and Tasseron, 1968). Theoretically, therefore, prostaglandin $E_2$ would appear to be the drug of choice for induction of labour.

Analgesia in labour may conveniently be obtained by the epidural route. Prophylactic shortening of the second stage of labour, as for any patient with heart disease, is advised.

It is generally recommended that oxytocics be withheld during the third stage of labour due to their potential vasoconstrictive activity, and used only in emergency.

Caesarean section is not indicated unless obstetric indications supervene. Only 12 of the previously reported patients were delivered by this means.

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# BOOK REVIEWS

**Year Book of Obstetrics and Gynaecology.** Edited by J. P. Greenhill. Pp. 442. Lloyd-Luke, London, 1974. Price: £9.10.

There is no more welcome or valued flower in the gynaecological garden than Greenhill's annual. The 1974 Year Book covers literature reviewed up to November 1973 and includes invited articles on acupuncture in surgery, obstetrics and gynaecology, on medical aspects of family planning, and on intrauterine progesterone for contraception. Papers on breech presentation and delivery and on the possible relationship between vaginal malignancy in the teenager and antecedent maternal oestrogen therapy are of special current interest. Editorial comments are, as ever, pungent. They contrive to combine a welcome for innovations in management and technique with a caution against premature acceptance to the detriment of sound clinical judgement. As a key to postgraduate education the Year Book has no rival. *S. Bender*

**Size at Birth.** Edited by Ciba Foundation Symposium 27 (New Series). Pp. ix+408 with figs. and tables. Elsevier, Excerpta Medica, Amsterdam, 1974. Price: £12.

Twenty-five years ago, a perinatal mortality rate of 20 per 1000 sounded hopelessly idealistic; but we are nearly there in Britain and a few other countries are even lower. Obstetricians and paediatricians are therefore more concerned with the preservation of quality than with the saving of life; and one of the best indicators of "quality" is a satisfactory size at birth. We have learned a great deal about the statistical relationships of birth size—for example, with maternal stature, parity, smoking habits and disorders such as diabetes and hypertension. But we still know remarkably little about the factors which control the rate of fetal growth. This volume, which contains sixteen papers, and the discussion, from a symposium held in London in March 1974, offers a useful summary of current knowledge and theories. Peter Gruenwald (Philadelphia) undermines the concept of "placental insufficiency" by arguing that the cause of fetal deprivation lies in factors which impair the supply line as a whole, more often than pathology in the placenta itself. Rodney Shearman (Sydney) describes endocrine and other influences. Victor Dubowitz (Hammersmith) reviews advances in the management of "the infant of inappropriate size" and describes some of the hazards of follow-up studies. Paul Polani (Guy's Hospital) reckons that less than half the variation of birthweight is genetic, and describes the mechanisms. Bengt Persson (Stockholm) discusses the metabolic control of diabetic pregnancies which, he says, have resulted in low mortality and morbidity rates and a "normalization" of the infant's size at birth. Stuart Campbell (Queen Charlotte's) reviews recent advances in measuring fetal growth by sonar. F. J. Schulte (Göttingen) describes neurological impairment in the small-for-dates infants of toxaemic mothers. Heird and his colleagues (New York) deal with parenteral nutrition of babies weighing less than 1200 g. at birth. Jean-Pierre Habicht (Guatemala) shows convincingly that improved feeding increases birth weights of infants born to mothers from an impoverished Latin-American community, and argues that reducing the incidence of low birthweight babies may be the best way of lowering infant mortality. Neville Butler (Bristol) reviews risk factors in intrauterine growth retardation. These papers on man are backed by reviews of work on animals by Elsie Widdowson (Cambridge), Mary Avery (Boston), D. E. Hill (Toronto), G. C. Liggins (Auckland), G. D. Thorburn (Oxford) and G. Alexander (New South Wales). In short, this is an informative and interesting volume which displays the problems, even if it shows that the solutions require further research. The book is beautifully printed.

*A. M. Thomson*

**The Placenta: Biological and Clinical Aspects.** By Kamran S. Moghissi and E. S. E. Hafez. Pp. xvi+406, with figs. and tables. Charles C. Thomas, Illinois, 1974. Price: $29.50.

This volume consists of the proceedings of a symposium on biological and clinical aspects of the placenta. As such, it is a useful collection of papers covering a substantial number of topics. As a book, it suffers from having 29 different authors with corresponding variations in style. The book is divided into four major sections which consist of sections on placental structure, transport and exchange mechanisms, metabolism and endocrine aspects and clinical aspects. The individual sections are by no means comprehensive and at times the result seems rather fragmentary. For example, the first section on structure of the placenta consists of two chapters entirely devoted to electron microscopic studies of placental cellular structure and scanning electron microscopy of terminal villi and amnion. In the section on transfer and exchange mechanisms, there is an excellent account of placental transfer of carbon dioxide and the chapter on protein transport throws light into a difficult and confusing field. However, it is strange that the question of fatty acid transfer and metabolism in the fetus is completely omitted, or perhaps this is a reflection on the paucity of work in this field at the present time. The section on metabolic and endocrine aspects contains useful and interesting chapters on placental lactogen and prolactin secretion, chorionic gonadotrophin and human chorionic thyrotrophin. The section on the clinical assessment of placental function is very limited; indeed, the emphasis on the oxytocin challenge test as a placental function test would not receive widespread enthusiasm. This book is too uneven in its content to be a generally useful or coherent account of modern concepts of placental function. It has relatively little to offer the clinician but does contain sections which are useful both for those who have lectures to prepare on placental function and those involved in research into various aspects of the placenta.

*E. M. Symonds*

## BOOKS RECEIVED BUT NOT REVIEWED

**Experiments in the Rehabilitation of the Mentally Handicapped.** Edited by H. C. GUNZBURG. Pp. xv+336, with 3 figs. and 59 tables. Published by The Butterworth Group, London, 1974. Price: £5.80.

**Clinical Chemistry. Conversion Scales for SI Units with Adult Normal (Reference) Values.** By A. M. BOLD and P. WILDING. Pp. 46. Published by Blackwell Scientific Publications, Oxford. Price: 80p.

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# PAPERS PRESENTED AT A MEETING OF THE BLAIR BELL SOCIETY AT ST. MARY'S HOSPITAL, LONDON ON 8th FEBRUARY, 1975

## THE RELEASE OF ARGININE VASOPRESSIN IN THE FETUS

BY
M. L. FORSLING
D. P. ALEXANDER
AND
H. G. BRITTON

ARGININE vasopressin (AVP) release was studied in fetal sheep. Haemorrhage, hypoxia and raised cerebral arterial osmotic pressure increased circulating AVP in the acute preparation; this was not due to slow clearance from the fetus. Chronic preparations had low fetal AVP levels until a few days before term when a moderate rise occurred.

## THE QUANTITATION OF UTERINE ACTIVITY

BY
P. J. STEER
J. WOOLFSON
D. LITTLE
D. ANTEBI
M. KELLY
N. LEWIS
AND
R. W. BEARD

TWENTY normal patients were studied in oxytocin-induced labour. Maximum intrauterine pressure above baseline multiplied by duration of contractions summated per half hour distinguished three phases of labour. During the first there was a steady increase in uterine activity (incremental phase): during the second (stable phase) uterine activity altered little even if oxytocin infusion was increased and the third phase occupied the second stage of labour.

## CELL MEDIATED IMMUNITY IN THE STUDY OF THE FETO-MATERNAL RELATIONSHIP

BY
G. M. STIRRAT

PREGNANCY failed to induce consistent cell-mediated immunity to paternal antigens in mice. This was demonstrated by the action of maternal lymph node cells against paternal fibroblasts. There was a spectrum of responses by the pregnant animals, possibly due to sensitization in some, sensitization with blocking of effector response in others and the remainder having lymph node cell activity suppressed by pregnancy.

## METABOLIC PROFILES IN DIABETIC PREGNANCY

BY
N. W. OAKLEY
M. M. D. GILLMER
F. M. BROOKE
AND
R. W. BEARD

MILD chemical diabetics showed close correlation between mean maternal diurnal glucose and glucose tolerance test area, and also a close correlation between these functions and glucose assimilation during the first two hours jo neonatal life. Studies on insulin-requiring diabetics showed that unsuspected morning hyperglycaemia and nocturnal hypoglycaemia may often occur, and suggest that glucose profiles may have a useful role in the attainment of optimum control in such patients.

## THE RESULTS OF INTRAPARTUM FETAL MONITORING

BY

P. T. EDINGTON

AND

R. W. BEARD

A GROUP of 251 patients with complicated pregnancies were studied during labour; 21·5 per cent had suspicious fetal heart rate (FHR) traces and 6·9 per cent had a fetal blood pH of 7·25 or less. In 555 normal pregnancies, 15·8 per cent of patients had suspicious FHR traces in labour and 4·9 per cent had fetal pH of 7·25 or less. This high incidence of fetal acidosis indicates that all patients should be similarly monitored in labour. A further 158 patients had fetal blood sampling because of an abnormal FHR trace; 28 per cent had fetal pH of 7·25 or less, indicating that continuous FHR recording must be combined with fetal blood sampling for accurate detection of fetal acidosis.

## FUNCTIONALLY ORIENTATED DIAGNOSIS OF AMENORRHOEA IN 45 CONSECUTIVE CASES

BY

M. G. R. HULL

S. FRANKS

A. S. MCNEILLY

M. A. F. MURRAY

AND

H. S. JACOBS

THIRTEEN of the patients studied had ovarian failure, hypogonadotrophic hypogonadism or a pituitary tumour. Five of the remaining 32 women had hyperprolactinaemia. Almost half of the other 27 had a psychiatric disorder generally associated with negative responses to progestogen and clomiphene stimulation, and impaired releasing hormone tests. Those without psychiatric disorders were highly sensitive to clomiphene. Amenorrhoea following oral contraception did not seem to be a specific entity.

## THE INCIDENCE, SIGNIFICANCE AND TREATMENT OF HYPERPROLACTINAEMIA AND AMENORRHOEA

BY

S. FRANKS

M. A. F. MURRAY

A. M. JEQUIER

S. J. STEELE

J. D. N. NABARRO

AND

H. S. JACOBS

PROLACTIN levels were normal in serum from 7 women with primary amenorrhoea, 13 with weight related disorders, 10 with systemic disorders and 14 with post oral contraceptive amenorrhoea. Prolactin was elevated in 8 out of 40 patients with functional secondary amenorrhoea and in 12 with pituitary tumours; only 6 of these patients had galactorrhoea. Bromocriptine (Sandoz) reduced prolactin secretion in all patients with hyperprolactinaemia and produced ovulatory menstruation in two.

## CONTROL OF FSH SECRETION IN MEN

BY

F. J. BRAMBLE

A. C. BROUGHTON

S. ECCLES

M. A. F. MURRAY

AND

H. S. JACOBS

THE serum concentrations of FSH, LH and testosterone (and their response to chorionic gonadotrophin or exogenous releasing hormone) were studied in a larger number of men with testicular disorders. The results indicated that inhibin (a postulated non-androgenic testicular hormone) modulated FSH secretion at a pituitary rather than a hypothalamic site. FSH levels were raised after removal of testes but not after other surgery or when a cryptorchid testis was removed, indicating that testosterone has no role in the regulation of FSH secretion.

# CONTROL OF ANDROGEN SECRETION IN WOMEN

BY
H. S. JACOBS
A. E. RIPPON
AND
V. H. T. JAMES

ANDROGEN production was studied in three women with polycystic ovaries and two with idiopathic hirsutism. Hirsute patients had excessive production of androstenedione and testosterone. However, androgen levels were only partly suppressable by glucocorticoid, suggesting production mainly by the ovary. Disparate testosterone and cortisol levels, and suppression by dexamethasone in one patient with idiopathic hirsutism was consistent with a diagnosis of glucocorticoid suppressable ovarian overproduction of androgens.

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